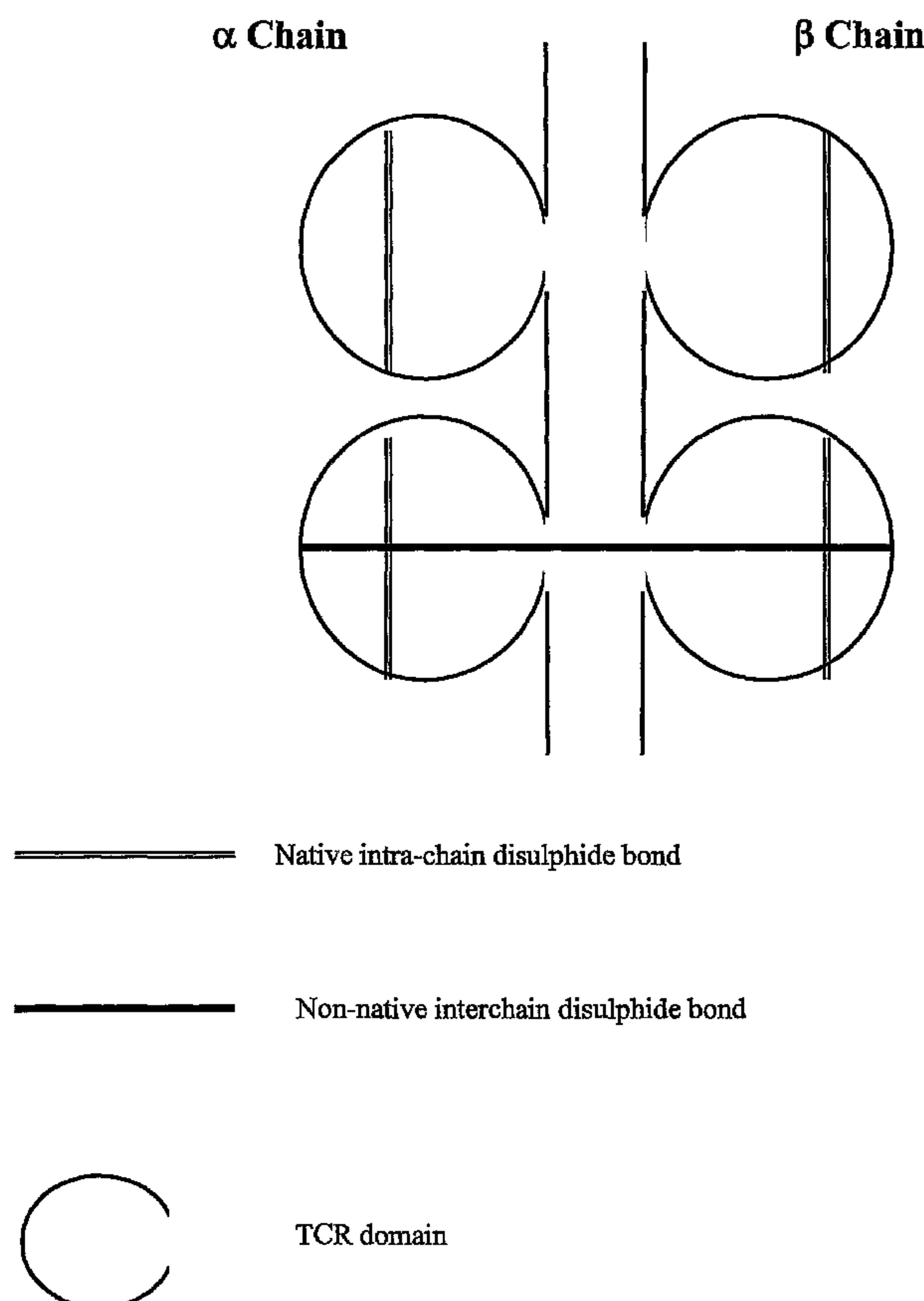




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(57) **Abrégé/Abstract:**

The present invention provides a soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof. (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR.

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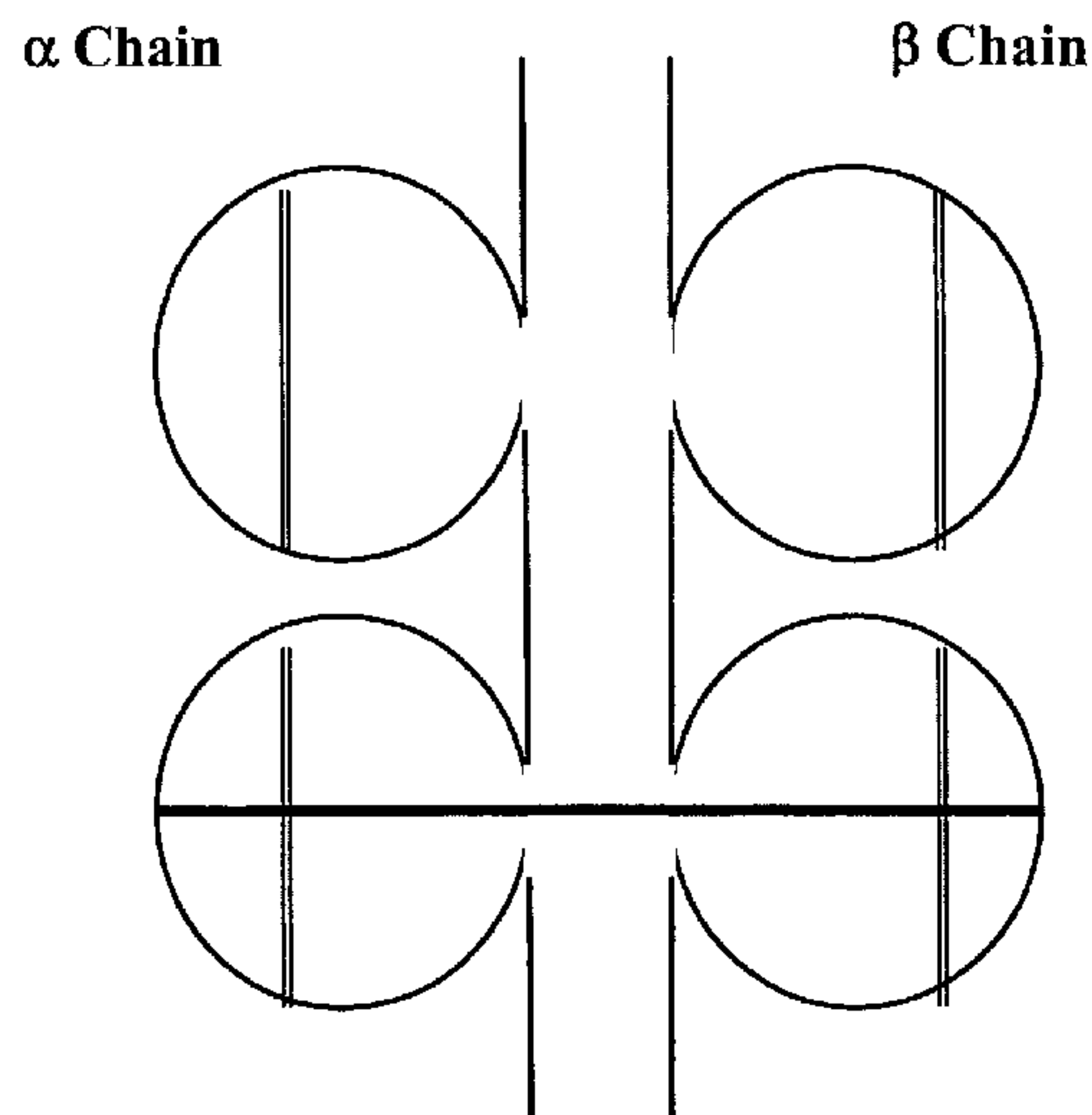
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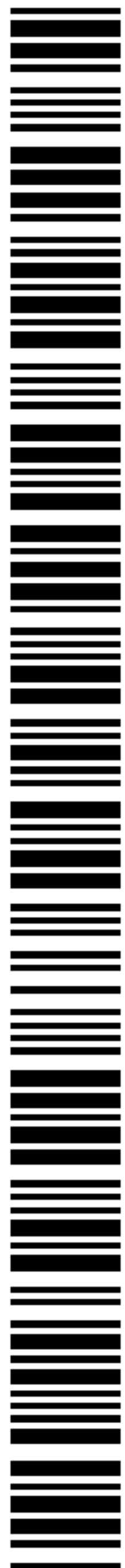
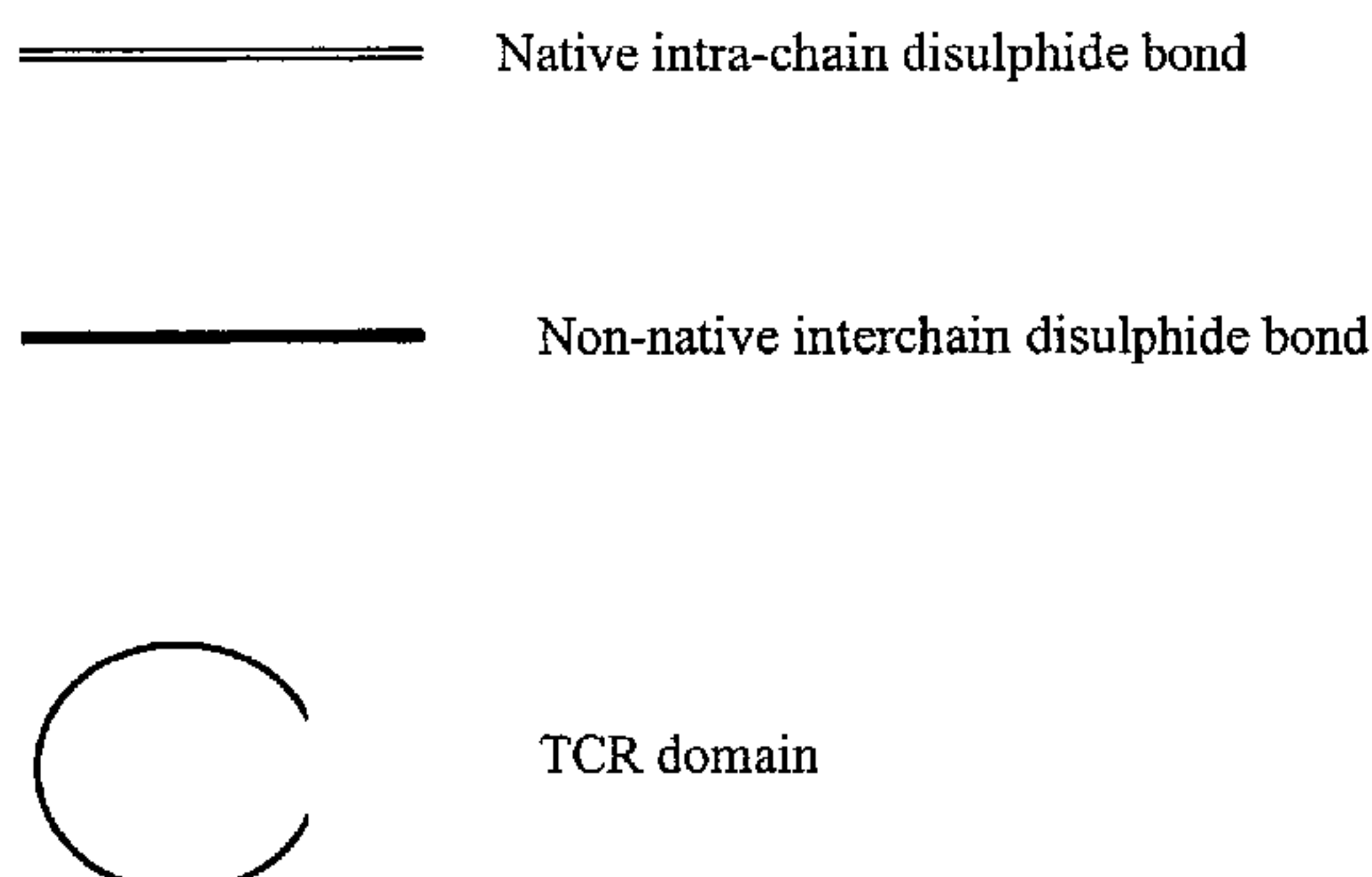
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(54) Title: SOLUBLE T CELL RECEPTOR



(57) Abstract: The present invention provides a soluble T cell receptor (sTCR), which comprises (i) all for part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof. (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR.



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SOLUBLE T CELL RECEPTOR

The present invention relates to soluble T cell receptors (TCRs).

5 As is described in WO 99/60120, TCRs mediate the recognition of specific Major Histocompatibility Complex (MHC)-peptide complexes by T cells and, as such, are essential to the functioning of the cellular arm of the immune system.

10 Antibodies and TCRs are the only two types of molecules which recognise antigens in a specific manner, and thus the TCR is the only receptor for particular peptide antigens presented in MHC, the alien peptide often being the only sign of an abnormality within a cell. T cell recognition occurs when a T-cell and an antigen presenting cell (APC) are in direct physical contact, and is initiated by ligation of antigen-specific TCRs with pMHC complexes.

15

The TCR is a heterodimeric cell surface protein of the immunoglobulin superfamily which is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. TCRs exist in $\alpha\beta$ and $\gamma\delta$ forms, which are structurally similar but T cells expressing them have quite distinct anatomical locations and probably
20 functions. The extracellular portion of the receptor consists of two membrane-proximal constant domains, and two membrane-distal variable domains bearing polymorphic loops analogous to the complementarity determining regions (CDRs) of antibodies. It is these loops which form the binding site of the TCR molecule and determine peptide specificity. The MHC class I and class II ligands are also
25 immunoglobulin superfamily proteins but are specialised for antigen presentation, with a polymorphic peptide binding site which enables them to present a diverse array of short peptide fragments at the APC cell surface.

Soluble TCRs are useful, not only for the purpose of investigating specific TCR-
30 pMHC interactions, but also potentially as a diagnostic tool to detect infection, or to detect autoimmune disease markers. Soluble TCRs also have applications in staining, for example to stain cells for the presence of a particular peptide antigen presented in

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the context of the MHC. Similarly, soluble TCRs can be used to deliver a therapeutic agent, for example a cytotoxic compound or an immunostimulating compound, to cells presenting a particular antigen. Soluble TCRs may also be used to inhibit T cells, for example, those reacting to an auto-immune peptide antigen.

5

Proteins which are made up of more than one polypeptide subunit and which have a transmembrane domain can be difficult to produce in soluble form because, in many cases, the protein is stabilised by its transmembrane region. This is the case for the TCR, and is reflected in the scientific literature which describes truncated forms of TCR, containing either only extracellular domains or extracellular and cytoplasmic domains, which can be recognised by TCR-specific antibodies (indicating that the part of the recombinant TCR recognised by the antibody has correctly folded), but which cannot be produced at a good yield, which are not stable at low concentrations and/or which cannot recognise MHC-peptide complexes. This literature is reviewed in WO 99/60120.

15

A number of papers describe the production of TCR heterodimers which include the native disulphide bridge which connects the respective subunits (Garboczi, *et al.*, (1996), *Nature* **384**(6605): 134-41; Garboczi, *et al.*, (1996), *J Immunol* **157**(12): 5403-10; Chang *et al.*, (1994), *PNAS USA* **91**: 11408-11412; Davodeau *et al.*, (1993), *J. Biol. Chem.* **268**(21): 15455-15460; Golden *et al.*, (1997), *J. Imm. Meth.* **206**: 163-169; US Patent No. 6080840). However, although such TCRs can be recognised by TCR-specific antibodies, none were shown to recognise its native ligand at anything other than relatively high concentrations and/or were not stable.

25

In WO 99/60120, a soluble TCR is described which is correctly folded so that it is capable of recognising its native ligand, is stable over a period of time, and can be produced in reasonable quantities. This TCR comprises a TCR α or γ chain extracellular domain dimerised to a TCR β or δ chain extracellular domain respectively, by means of a pair of C-terminal dimerisation peptides, such as leucine zippers. This strategy of producing TCRs is generally applicable to all TCRs.

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Reiter *et al*, *Immunity*, 1995, 2:281-287, details the construction of a soluble molecule comprising disulphide-stabilised TCR α and β variable domains, one of which is linked to a truncated form of *Pseudomonas* exotoxin (PE38). One of the stated reasons for producing this molecule was to overcome the inherent instability of single-chain TCRs. The position of the novel disulphide bond in the TCR variable domains was identified via homology with the variable domains of antibodies, into which these have previously been introduced (for example see Brinkmann, *et al.* (1993), *Proc. Natl. Acad. Sci. USA* 90: 7538-7542, and Reiter, *et al.* (1994) *Biochemistry* 33: 5451-5459). However, as there is no such homology between antibody and TCR constant domains, such a technique could not be employed to identify appropriate sites for new inter-chain disulphide bonds between TCR constant domains.

Given the importance of soluble TCRs, it would be desirable to provide an alternative way of producing such molecules.

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Various embodiments of this invention provide a soluble $\alpha\beta$ -form T cell receptor (sTCR), wherein a covalent disulphide bond links a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain, wherein an interchain disulphide bond in native TCR is not present.

20

Various embodiments of this invention provide a soluble $\alpha\beta$ -form T cell receptor (sTCR), wherein a covalent disulphide bond links cysteine residues substituted for: Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01; Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01; Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01; Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01; or Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01.

Various embodiments of this invention provide a soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant

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domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR and wherein an interchain disulphide bond in native TCR is not present.

- 5 Various embodiments of this invention provide a soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, characterised in that (i) and (ii) are linked by a disulphide bond
 10 between cysteine residues substituted for: Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01; Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01; Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01; Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01; or Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of
 15 TRBC1*01 or TRBC2*01.

Various embodiments of this invention provide a multivalent T cell receptor (TCR) complex comprising a plurality of sTCRs of this invention, including a sTCR multimer.

- 20 Various embodiments of this invention provide a method for detecting MHC-peptide complexes, which comprises: (i) providing a soluble TCR of this invention or a multivalent T cell receptor complex of this invention; (ii) contacting the soluble TCR or multivalent TCR complex with the MHC-peptide complexes; and (iii) detecting binding of the soluble TCR or multivalent TCR complex to the MHC-peptide complexes.

25

- Various embodiments of this invention provide a nucleic acid molecule comprising a sequence encoding a TCR α chain or a TCR β chain of a sTCR of this invention, or a sequence complementary thereto. Also provided is a vector comprising such a nucleic acid molecule as well as host cells comprising such a vector. Also provided is a method
 30 for obtaining such a chain comprising incubating the aforementioned host cell under conditions causing expression of the peptide, then purifying the peptide.

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Various embodiments of this invention provide a method for obtaining a soluble T cell receptor (sTCR), which method comprises: incubating a host cell which comprises a vector comprising a nucleic acid molecule encoding (i) all or part of a TCR α chain, except the transmembrane domain thereof, and a host cell which comprises a vector comprising a
5 nucleic acid molecule encoding (ii) all or part of a TCR β chain, except the transmembrane domain thereof under conditions causing expression of (i) and (ii), wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain; purifying (i) and (ii); and mixing (i) and (ii) under refolding conditions such that they are linked by a disulphide bond between constant domain residues which is not
10 present in native TCR.

Various embodiments of this invention provide a method for obtaining a soluble $\alpha\beta$ -form T cell receptor (sTCR), which method comprises: incubating a host cell which comprises a vector comprising a nucleic acid molecule encoding a TCR α chain and a host cell which
15 comprises a vector comprising a nucleic acid molecule encoding a TCR β chain under conditions causing expression of the respective TCR chains; purifying the respective TCR chains; and mixing the respective TCR chains under refolding conditions such that a covalent disulphide bond links a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β
20 chain.

Various embodiments of this invention provide a complex of this invention, wherein the sTCRs or sTCR multimers are present in a lipid bilayer or are attached to a particle.

25 Various embodiments of this invention provide a method for detecting MHC-peptide complexes, which comprises: (i) providing a soluble TCR produced by a method of this invention or a multivalent T cell receptor complex produced by a method of this invention; (ii) contacting the soluble TCR or multivalent TCR complex with the MHC-peptide complexes; and (iii) detecting binding of the soluble TCR or multivalent TCR complex to
30 the MHC-peptide complexes.

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According to a first aspect, the present invention provides a soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR.

In another aspect, the invention provides a soluble $\alpha\beta$ -form T cell receptor (sTCR), wherein a covalent disulphide bond links a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain.

The sTCRs of the present invention have the advantage that they do not contain heterologous polypeptides which may be immunogenic, or which may result in the sTCR being cleared quickly from the body. Furthermore, TCRs of the present invention have a three-dimensional structure which is highly similar to the native TCRs from which they are derived and, due to this structural similarity, they are not

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likely to be immunogenic. sTCRs in accordance with the invention may be for recognising Class I MHC-peptide complexes or Class II MHC-peptide complexes.

TCRs of the present invention are soluble. In the context of this application, solubility
5 is defined as the ability of the TCR to be purified as a mono disperse heterodimer in phosphate buffered saline (PBS) (KCL 2.7mM, KH₂PO₄ 1.5mM, NaCl 137mM and Na₂PO₄ 8mM, pH 7.1-7.5. Life Technologies, Gibco BRL) at a concentration of 1mg/ml and for >90% of said TCR to remain as a mono disperse heterodimer after incubation at 25 °C for 1 hour. In order to assess the solubility of the TCR, it is first
10 purified as described in Example 2. Following this purification, 100µg of the TCR is analysed by analytical size exclusion chromatography e.g. using a Pharmacia Superdex 75 HR column equilibrated in PBS. A further 100µg of the TCR is incubated at 25°C for 1 hour and then analysed by size exclusion chromatography as before. The size exclusion traces are then analysed by integration and the areas under
15 the peaks corresponding to the mono disperse heterodimer are compared. The relevant peaks may be identified by comparison with the elution position of protein standards of known molecular weight. The mono disperse heterodimeric soluble TCR has a molecular weight of approximately 50 kDa. As stated above, the TCRs of the present invention are soluble. However, as explained in more detail below, the TCRs can be
20 coupled to a moiety such that the resulting complex is insoluble, or they may be presented on the surface of an insoluble solid support.

The numbering of TCR amino acids used herein follows the IMGT system described in The T Cell Receptor Factsbook, 2001, LeFranc & LeFranc, Academic Press. In this
25 system, the α chain constant domain has the following notation: TRAC*01, where “TR” indicates T Cell Receptor gene; “A” indicates α chain gene; C indicates constant region; and “*01” indicates allele 1. The β chain constant domain has the following notation: TRBC1*01. In this instance, there are two possible constant region genes “C1” and “C2”. The translated domain encoded by each allele can be made up from
30 the genetic code of several exons; therefore these are also specified. Amino acids are numbered according to the exon of the particular domain in which they are present.

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The extracellular portion of native TCR consists of two polypeptides ($\alpha\beta$ or $\gamma\delta$) each of which has a membrane-proximal constant domain, and a membrane-distal variable domain (see Figure 1). Each of the constant and variable domains includes an intra-chain disulphide bond. The variable domains contain the highly polymorphic loops analogous to the complementarity determining regions (CDRs) of antibodies. CDR3 of the TCR interacts with the peptide presented by MHC, and CDRs 1 and 2 interact with the peptide and the MHC. The diversity of TCR sequences is generated via somatic rearrangement of linked variable (V), diversity (D), joining (J), and constant genes. Functional α chain polypeptides are formed by rearranged V-J-C regions, whereas β chains consist of V-D-J-C regions. The extracellular constant domain has a membrane proximal region and an immunoglobulin region. The membrane proximal region consists of the amino acids between the transmembrane domain and the membrane proximal cysteine residue. The constant immunoglobulin domain consists of the remainder of the constant domain amino acid residues, extending from the membrane proximal cysteine to the beginning of the joining region, and is characterised by the presence of an immunoglobulin-type fold. There is a single α chain constant domain, known as C α 1 or TRAC*01, and two different β constant domains, known as C β 1 or TRBC1*01 and C β 2 or TRBC2*01. The difference between these different β constant domains is in respect of amino acid residues 4, 5 and 37 of exon 1. Thus, TRBC1*01 has 4N, 5K and 37F in exon 1 thereof, and TRBC2*01 has 4K, 5N and 37Y in exon 1 thereof. The extent of each of the TCR extracellular domains is somewhat variable.

In the present invention, the disulphide bond is introduced between residues located in the constant domains (or parts thereof) of the respective chains. The respective chains of the TCR comprise sufficient of the variable domains thereof to be able to interact with its pMHC complex. Such interaction can be measured using a BIAcore 3000™ or BIAcore 2000™ instrument as described in Example 3 herein or in WO99/6120 respectively.

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In one embodiment, the respective chains of the sTCR of the invention also comprise the intra-chain disulphide bonds thereof. The TCR of the present invention may comprise all of the extracellular constant Ig region of the respective TCR chains, and preferably all of the extracellular domain of the respective chains, i.e. including the membrane proximal region. In native TCR, there is a disulphide bond linking the conserved membrane proximal regions of the respective chains. In one embodiment of the present invention, this disulphide bond is not present. This may be achieved by mutating the appropriate cysteine residues (amino acid 4, exon 2 of the TRAC*01 gene and amino acid 2 of both the TRBC1*01 and TRBC2*01 genes respectively) to another amino acid, or truncating the respective chains so that the cysteine residues are not included. A preferred soluble TCR according to the invention comprises the native α and β TCR chains truncated at the C-terminus such that the cysteine residues which form the native interchain disulphide bond are excluded, i.e. truncated at the residue 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues N-terminal to the cysteine residues. It is to be noted however that the native inter-chain disulphide bond may be present in TCRs of the present invention, and that, in certain embodiments, only one of the TCR chains has the native cysteine residue which forms the native interchain disulphide bond. This cysteine can be used to attach moieties to the TCR.

However, the respective TCR chains may be shorter. Because the constant domains are not directly involved in contacts with the peptide-MHC ligands, the C-terminal truncation point may be altered substantially without loss of functionality.

Alternatively, a larger fragment of the constant domains may be present than is preferred herein, i.e. the constant domains need not be truncated just prior to the cysteines forming the interchain disulphide bond. For instance, the entire constant domain except the transmembrane domain (i.e. the extracellular and cytoplasmic domains) could be included. It may be advantageous in this case to mutate one or more of the cysteine residues forming the interchain disulphide bond in the cellular TCR to another amino acid residue which is not involved in disulphide bond formation, or to delete one or more of these residues.

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The signal peptide may be omitted if the soluble TCR is to be expressed in prokaryotic cells, for example *E.coli*, since it does not serve any purpose in the mature TCR for its ligand binding ability, and may in some circumstances prevent the formation of a functional soluble TCR. In most cases, the cleavage site at which the signal peptide is removed from the mature TCR chains is predicted but not experimentally determined. Engineering the expressed TCR chains such that they are a few, i.e. up to about 10 for example, amino acids longer or shorter at the N-terminal end may have no significance for the functionality (i.e. the ability to recognise pMHC) of the soluble TCR. Certain additions which are not present in the original protein sequence could be added. For example, a short tag sequence which can aid in purification of the TCR chains could be added, provided that it does not interfere with the correct structure and folding of the antigen binding site of the TCR.

For expression in *E.coli*, a methionine residue may be engineered onto the N-terminal starting point of the predicted mature protein sequence in order to enable initiation of translation.

Far from all residues in the variable domains of TCR chains are essential for antigen specificity and functionality. Thus, a significant number of mutations can be introduced in this domain without affecting antigen specificity and functionality. Far from all residues in the constant domains of TCR chains are essential for antigen specificity and functionality. Thus, a significant number of mutations can be introduced in this region without affecting antigen specificity.

The TCR β chain contains a cysteine residue which is unpaired in the cellular or native TCR. It is preferred if this cysteine residue is removed or mutated to another residue to avoid incorrect intrachain or interchain pairing. Substitutions of this cysteine residue for another residue, for example serine or alanine, can have a significant positive effect on refolding efficiencies *in vitro*.

The disulphide bond may be formed by mutating non-cysteine residues on the respective chains to cysteine, and causing the bond to be formed between the mutated

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residues. Residues whose respective β carbons are approximately 6 Å (0.6 nm) or less, and preferably in the range 3.5 Å (0.35 nm) to 5.9 Å (0.59 nm) apart in the native TCR are preferred, such that a disulphide bond can be formed between cysteine residues introduced in place of the native residues. It is preferred if the disulphide bond is between residues in the constant immunoglobulin region, although it could be between residues of the membrane proximal region. Preferred sites where cysteines can be introduced to form the disulphide bond are the following residues in exon 1 of TRAC*01 for the TCR α chain and TRBC1*01 or TRBC2*01 for the TCR β chain:

TCR α chain	TCR β chain	Native β carbon separation (nm)
Thr 48	Ser 57	0.473
Thr 45	Ser 77	0.533
Tyr 10	Ser 17	0.359
Thr 45	Asp 59	0.560
Ser 15	Glu 15	0.59

10

One sTCR of the present invention is derived from the A6 Tax TCR (Garboczi *et al*, *Nature*, 1996, 384(6605): 134-141). In one embodiment, the sTCR comprises the whole of the TCR α chain which is N-terminal of exon 2, residue 4 of TRAC*01 (amino acid residues 1-182 of the α chain according to the numbering used in Garboczi *et al*) and the whole of the TCR β chain which is N-terminal of exon 2, residue 2 of both TRBC1*01 and TRBC2*01 (amino acid residues 1-210 of the β chain according to the numbering used in Garboczi *et al*). In order to form the disulphide bond, threonine 48 of exon 1 in TRAC*01 (threonine 158 of the α chain according to the numbering used in Garboczi *et al*) and serine 57 of exon 1 in both TRBC1*01 and TRBC2*01 (serine 172 of the β chain according to the numbering used in Garboczi *et al*) may each be mutated to cysteine. These amino acids are located in β strand D of the constant domain of α and β TCR chains respectively.

It is to be noted that, in Figures 3a and 3b, residue 1 (according to the numbering used in Garboczi *et al*) is K and N respectively. The N-terminal methionine residue is not present in native A6 Tax TCR and, as mentioned above, is sometimes present when the respective chains are produced in bacterial expression systems.

25

Now that the residues in human TCRs which can be mutated into cysteine residues to form a new interchain disulphide bond have been identified, those of skill in the art will be able to mutate any TCR in the same way to produce a soluble form of that TCR having a new interchain disulphide bond. In humans, the skilled person merely needs to look for the following motifs in the respective TCR chains to identify the residue to be mutated (the shaded residue is the residue for mutation to a cysteine).

10	α Chain Thr 48:	DSDVYITDKTVLDMRSMDFK (amino acids 39-58 of exon 1 of the TRAC*01 gene)
	α Chain Thr 45:	QSKDSDVYIDKTVLDMRSM(amino acids 36-55 of exon 1 of the TRAC*01 gene)
15	α Chain Tyr 10:	DIQNPDPAVYQLRDSKSSDK(amino acids 1-20 of exon 1 of the TRAC*01 gene)
20	α Chain Ser 15:	DPAVYQLRDSKSSDKSVCLF(amino acids 6-25 of exon 1 of the TRAC*01 gene)
	β Chain Ser 57:	NGKEVHSGVSTDQPQLKEQP(amino acids 48- 67 of exon 1 of the TRBC1*01 & TRBC2*01 genes)
25	β Chain Ser 77:	ALNDSRYALSSRLRVSATFW(amino acids 68- 87 of exon 1 of the TRBC1*01 & TRBC2*01 genes)
	β Chain Ser 17:	PPEVAVFEPSEAEISHTQKA(amino acids 8- 27 of exon 1 of the TRBC1*01 & TRBC2*01 genes)
30	β Chain Asp 59:	KEVHSGVSTDQPQLKEQPAL(amino acids 50- 69 of exon 1 of the TRBC1*01 & TRBC2*01 genes gene)

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β Chain Glu 15: VFPPEVAVFEPSEAEISHTQ(amino acids 6- 25 of exon 1 of the TRBC1*01 & TRBC2*01 genes)

In other species, the TCR chains may not have a region which has 100% identity to the above motifs. However, those of skill in the art will be able to use the above motifs to identify the equivalent part of the TCR α or β chain and hence the residue to be mutated to cysteine. Alignment techniques may be used in this respect. For example, ClustalW, available on the European Bioinformatics Institute website (<http://www.ebi.ac.uk/index.html>) can be used to compare the motifs above to a particular TCR chain sequence in order to locate the relevant part of the TCR sequence for mutation.

The present invention includes within its scope human disulphide-linked $\alpha\beta$ TCRs, as well as disulphide-linked $\alpha\beta$ TCRs of other mammals, including, but not limited to, mouse, rat, pig, goat and sheep. As mentioned above, those of skill in the art will be able to determine sites equivalent to the above-described human sites at which cysteine residues can be introduced to form an inter-chain disulphide bond. For example, the following shows the amino acid sequences of the mouse C α and C β soluble domains, together with motifs showing the murine residues equivalent to the human residues mentioned above that can be mutated to cysteines to form a TCR interchain disulphide bond (where the relevant residues are shaded):

Mouse C α soluble domain:

PYIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMKAMDS
KSNGAIAWSNQTSTFCQDIFKETNATYPSSDVP

Mouse C β soluble domain:

EDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSWWVNGREVVHSGVST
DPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDKWPEGSPKPVTQN
ISAEAWGRAD

Murine equivalent of human α Chain Thr 48: ESGTFITDKTVLDMKAMDSK

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Murine equivalent of human α Chain Thr 45: KTMESGTFI~~II~~DKTVLDMKAM

Murine equivalent of human α Chain Tyr 10: YIQNPEPAVY~~Y~~QLKDPRSQDS

5

Murine equivalent of human α Chain Ser 15: AVYQLKDPRS~~S~~QDSTLCLFTD

Murine equivalent of human β Chain Ser 57: NGREVHSGV~~S~~TDPQAYKESN

10 Murine equivalent of human β Chain Ser 77: KESNYSYCL~~S~~SRLRVSATFW

Murine equivalent of human β Chain Ser 17: PPKVSLFEP~~S~~SKAEIANKQKA

Murine equivalent of human β Chain Asp 59: REVHSGVST~~D~~PQAYKESNYS

15

Murine equivalent of human β Chain Glu 15: VTPPKVSLF~~E~~PSKAEIANKQ

In a preferred embodiment of the present invention, (i) and (ii) of the TCR each comprise the functional variable domain of a first TCR fused to all or part of the constant domain of a second TCR, the first and second TCRs being from the same species and the inter-chain disulphide bond being between residues in said respective all or part of the constant domain not present in native TCR. In one embodiment, the first and second TCRs are human. In other words, the disulphide bond-linked constant domains act as a framework on to which variable domains can be fused. The resulting TCR will be substantially identical to the native TCR from which the first TCR is obtained. Such a system allows the easy expression of any functional variable domain on a stable constant domain framework.

The constant domains of the A6 Tax sTCR described above, or indeed the constant domains of any of the mutant $\alpha\beta$ TCRs having a new interchain disulphide bond described above, can be used as framework onto which heterologous variable domains

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can be fused. It is preferred if the fusion protein retains as much of the conformation of the heterologous variable domains as possible. Therefore, it is preferred that the heterologous variable domains are linked to the constant domains at any point between the introduced cysteine residues and the N terminus of the constant domain. For the

5 A6 Tax TCR, the introduced cysteine residues on the α and β chains are preferably located at threonine 48 of exon 1 in TRAC*01 (threonine 158 of the α chain according to the numbering used in Garboczi *et al*) and serine 57 of exon 1 in both TRBC1*01 and TRBC2*01 (serine 172 of the β chain according to the numbering used in

10 Garboczi *et al*) respectively. Therefore it is preferred if the heterologous α and β chain variable domain attachment points are between residues 48 (159 according to the numbering used in Garboczi *et al*) or 58 (173 according to the numbering used in Garboczi *et al*) and the N terminus of the α or β constant domains respectively.

The residues in the constant domains of the heterologous α and β chains

15 corresponding to the attachment points in the A6 Tax TCR can be identified by sequence homology. The fusion protein is preferably constructed to include all of the heterologous sequence N-terminal to the attachment point.

As is discussed in more detail below, the sTCR of the present invention may be

20 derivatised with, or fused to, a moiety at its C or N terminus. The C terminus is preferred as this is distal from the binding domain. In one embodiment, one or both of the TCR chains have a cysteine residue at its C and/or N terminus to which such a moiety can be fused.

25 A soluble TCR (which is preferably human) of the present invention may be provided in substantially pure form, or as a purified or isolated preparation. For example, it may be provided in a form which is substantially free of other proteins.

A plurality of soluble TCRs of the present invention may be provided in a multivalent

30 complex. Thus, the present invention provides, in one aspect, a multivalent T cell receptor (TCR) complex, which comprises a plurality of soluble T cell receptors as described herein. Each of the plurality of soluble TCRs is preferably identical.

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In another aspect, the invention provides a method for detecting MHC-peptide complexes which method comprises:

- (i) providing a soluble T cell receptor or a multivalent T cell receptor complex as described herein;
- (ii) contacting the soluble T cell receptor or multivalent TCR complex with the MHC-peptide complexes; and
- (iii) detecting binding of the soluble T cell receptor or multivalent TCR complex to the MHC-peptide complexes.

10

In the multivalent complex of the present invention, the TCRs may be in the form of multimers, and/or may be present on or associated with a lipid bilayer, for example, a liposome.

- 15 In its simplest form, a multivalent TCR complex according to the invention comprises a multimer of two or three or four or more T cell receptor molecules associated (e.g. covalently or otherwise linked) with one another, preferably via a linker molecule. Suitable linker molecules include, but are not limited to, multivalent attachment molecules such as avidin, streptavidin, neutravidin and extravidin, each of which has
20 four binding sites for biotin. Thus, biotinylated TCR molecules can be formed into multimers of T cell receptors having a plurality of TCR binding sites. The number of TCR molecules in the multimer will depend upon the quantity of TCR in relation to the quantity of linker molecule used to make the multimers, and also on the presence or absence of any other biotinylated molecules. Preferred multimers are dimeric,
25 trimeric or tetrameric TCR complexes.

Structures which are a good deal larger than TCR tetramers may be used in tracking or targeting cells expressing specific MHC-peptide complex. Preferably the structures are in the range 10nm to 10µm in diameter. Each structure may display multiple TCR
30 molecules at a sufficient distance apart to enable two or more TCR molecules on the structure to bind simultaneously to two or more MHC-peptide complexes on a cell and thus increase the avidity of the multimeric binding moiety for the cell.

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Suitable structures for use in the invention include membrane structures such as liposomes and solid structures which are preferably particles such as beads, for example latex beads. Other structures which may be externally coated with T cell
5 receptor molecules are also suitable. Preferably, the structures are coated with T cell receptor multimers rather than with individual T cell receptor molecules.

In the case of liposomes, the T cell receptor molecules or multimers thereof may be attached to or otherwise associated with the membrane. Techniques for this are well
10 known to those skilled in the art.

A label or another moiety, such as a toxic or therapeutic moiety, may be included in a multivalent TCR complex of the present invention. For example, the label or other moiety may be included in a mixed molecule multimer. An example of such a
15 multimeric molecule is a tetramer containing three TCR molecules and one peroxidase molecule. This could be achieved by mixing the TCR and the enzyme at a molar ratio of 3:1 to generate tetrameric complexes, and isolating the desired complex from any complexes not containing the correct ratio of molecules. These mixed molecules could contain any combination of molecules, provided that steric hindrance does not
20 compromise or does not significantly compromise the desired function of the molecules. The positioning of the binding sites on the streptavidin molecule is suitable for mixed tetramers since steric hindrance is not likely to occur.

Alternative means of biotinylating the TCR may be possible. For example, chemical
25 biotinylation may be used. Alternative biotinylation tags may be used, although certain amino acids in the biotin tag sequence are essential (Schatz, (1993).

Biotechnology N Y 11(10): 1138-43). The mixture used for biotinylation may also be varied. The enzyme requires Mg-ATP and low ionic strength, although both of these conditions may be varied e.g. it may be possible to use a higher ionic strength and a
30 longer reaction time. It may be possible to use a molecule other than avidin or streptavidin to form multimers of the TCR. Any molecule which binds biotin in a multivalent manner would be suitable. Alternatively, an entirely different linkage

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could be devised (such as poly-histidine tag to chelated nickel ion (Quiagen Product Guide 1999, Chapter 3 "Protein Expression, Purification, Detection and Assay" p. 35-37). Preferably, the tag is located towards the C-terminus of the protein so as to minimise the amount of steric hindrance in the interaction with peptide-MHC
5 complexes.

One or both of the TCR chains may be labelled with a detectable label, for example a label which is suitable for diagnostic purposes. Thus, the invention provides a method for detecting MHC-peptide complexes which method comprises contacting the MHC-peptide complexes with a TCR or multimeric TCR complex in accordance with the
10 invention which is specific for the MHC-peptide complex; and detecting binding of the TCR or multimeric TCR complex to the MHC-peptide complex. In tetrameric TCR formed using biotinylated heterodimers, fluorescent streptavidin (commercially available) can be used to provide a detectable label. A fluorescently-labelled tetramer
15 is suitable for use in FACS analysis, for example to detect antigen presenting cells carrying the peptide for which the TCR is specific.

Another manner in which the soluble TCRs of the present invention may be detected is by the use of TCR-specific antibodies, in particular monoclonal antibodies. There are
20 many commercially available anti-TCR antibodies, such as α F1 and β F1, which recognise the constant regions of the α and β chain, respectively.

The TCR (or multivalent complex thereof) of the present invention may alternatively or additionally be associated with (e.g. covalently or otherwise linked to) a therapeutic
25 agent which may be, for example, a toxic moiety for use in cell killing, or an immunostimulating agent such as an interleukin or a cytokine. A multivalent TCR complex of the present invention may have enhanced binding capability for a pMHC compared to a non-multimeric T cell receptor heterodimer. Thus, the multivalent TCR complexes according to the invention are particularly useful for tracking or targeting
30 cells presenting particular antigens *in vitro* or *in vivo*, and are also useful as intermediates for the production of further multivalent TCR complexes having such

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uses. The TCR or multivalent TCR complex may therefore be provided in a pharmaceutically acceptable formulation for use *in vivo*.

5 The invention also provides a method for delivering a therapeutic agent to a target cell, which method comprises contacting potential target cells with a TCR or multivalent TCR complex in accordance with the invention under conditions to allow attachment of the TCR or multivalent TCR complex to the target cell, said TCR or multivalent TCR complex being specific for the MHC-peptide complexes and having the therapeutic agent associated therewith.

10

In particular, the soluble TCR or multivalent TCR complex can be used to deliver therapeutic agents to the location of cells presenting a particular antigen. This would be useful in many situations and, in particular, against tumours. A therapeutic agent could be delivered such that it would exercise its effect locally but not only on the cell it binds to. Thus, one particular strategy envisages anti-tumour molecules linked to T cell receptors or multivalent TCR complexes specific for tumour antigens.

15

Many therapeutic agents could be employed for this use, for instance radioactive compounds, enzymes (perforin for example) or chemotherapeutic agents (cis-platin for example). To ensure that toxic effects are exercised in the desired location the toxin could be inside a liposome linked to streptavidin so that the compound is released slowly. This will prevent damaging effects during the transport in the body and ensure that the toxin has maximum effect after binding of the TCR to the relevant antigen presenting cells.

20

25

Other suitable therapeutic agents include:

- small molecule cytotoxic agents, i.e. compounds with the ability to kill mammalian cells having a molecular weight of less than 700 daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect.

30

Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, i.e. compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cis-platin,

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maytansine derivatives, rachelmycin, calicheamicin, docetaxel, etoposide, gemcitabine, ifosfamide, irinotecan, melphalan, mitoxantrone, sorfimer sodiumphotofrin II, temozolmide, topotecan, trimetreate glucuronate, auristatin E vincristine and doxorubicin;

- 5 • peptide cytotoxins, i.e. proteins or fragments thereof with the ability to kill mammalian cells. Examples include ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNAase and RNAase;
- radio-nuclides, i.e. unstable isotopes of elements which decay with the concurrent emission of one or more of α or β particles, or γ rays. Examples
10 include iodine 131, rhenium 186, indium 111, yttrium 90, bismuth 210 and 213, actinium 225 and astatine 213;
- prodrugs, such as antibody directed enzyme pro-drugs;
- immuno-stimulants, i.e. moieties which stimulate immune response. Examples
15 include cytokines such as IL-2, chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, etc, antibodies or fragments thereof, complement activators, xenogeneic protein domains, allogeneic protein domains,
20 viral/bacterial protein domains and viral/bacterial peptides.

Soluble TCRs or multivalent TCR complexes of the invention may be linked to an
20 enzyme capable of converting a prodrug to a drug. This allows the prodrug to be converted to the drug only at the site where it is required (i.e. targeted by the sTCR).

Examples of suitable MHC-peptide targets for the TCR according to the invention include, but are not limited to, viral epitopes such as HTLV-1 epitopes (e.g. the Tax
25 peptide restricted by HLA-A2; HTLV-1 is associated with leukaemia), HIV epitopes, EBV epitopes, CMV epitopes; melanoma epitopes (e.g. MAGE-1 HLA-A1 restricted epitope) and other cancer-specific epitopes (e.g. the renal cell carcinoma associated antigen G250 restricted by HLA-A2); and epitopes associated with autoimmune disorders, such as rheumatoid arthritis. Further disease-associated pMHC targets,
30 suitable for use in the present invention, are listed in the HLA Factbook (Barclay (Ed) Academic Press), and many others are being identified.

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A multitude of disease treatments can potentially be enhanced by localising the drug through the specificity of soluble TCRs.

5 Viral diseases for which drugs exist, e.g. HIV, SIV, EBV, CMV, would benefit from the drug being released or activated in the near vicinity of infected cells. For cancer, the localisation in the vicinity of tumours or metastasis would enhance the effect of toxins or immunostimulants. In autoimmune diseases, immunosuppressive drugs could be released slowly, having more local effect over a longer time-span while minimally affecting the overall immuno-capacity of the subject. In the prevention of
10 graft rejection, the effect of immunosuppressive drugs could be optimised in the same way. For vaccine delivery, the vaccine antigen could be localised in the vicinity of antigen presenting cells, thus enhancing the efficacy of the antigen. The method can also be applied for imaging purposes.

15 The soluble TCRs of the present invention may be used to modulate T cell activation by binding to specific pMHC and thereby inhibiting T cell activation. Autoimmune diseases involving T cell-mediated inflammation and/or tissue damage would be amenable to this approach, for example type I diabetes. Knowledge of the specific peptide epitope presented by the relevant pMHC is required for this use.

20

Medicaments in accordance with the invention will usually be supplied as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a patient). It may be provided
25 in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

The pharmaceutical composition may be adapted for administration by any appropriate
30 route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may

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be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Pharmaceutical compositions adapted for oral administration may be presented as
5 discrete units such as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions). Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-
10 solid, or liquid polyols etc.

For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

15 Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, 3(6):318 (1986). Pharmaceutical compositions adapted for topical administration may be
20 formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For infections of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be
25 formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

30

Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas. Pharmaceutical compositions adapted for nasal administration

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wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient. Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators. Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations. Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colourants, odourants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the substances of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used. The dosage may be repeated as often as appropriate. If side effects

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develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice.

Gene cloning techniques may be used to provide a sTCR of the invention, preferably in substantially pure form. These techniques are disclosed, for example, in J. Sambrook *et al Molecular Cloning* 2nd Edition, Cold Spring Harbor Laboratory Press (1989). Thus, in a further aspect, the present invention provides a nucleic acid molecule comprising a sequence encoding a chain of the soluble TCR of the present invention, or a sequence complementary thereto. Such nucleic acid sequences may be obtained by isolating TCR-encoding nucleic acid from T-cell clones and making appropriate mutations (by insertion, deletion or substitution).

The nucleic acid molecule may be in isolated or recombinant form. It may be incorporated into a vector and the vector may be incorporated into a host cell. Such vectors and suitable hosts form yet further aspects of the present invention.

The invention also provides a method for obtaining a TCR chain, which method comprises incubating such a host cell under conditions causing expression of the TCR chain and then purifying the polypeptide.

The soluble TCRs of the present invention may be obtained by expression in a bacterium such as *E. coli* as inclusion bodies, and subsequent refolding *in vitro*.

Refolding of the TCR chains may take place *in vitro* under suitable refolding conditions. In a particular embodiment, a TCR with correct conformation is achieved by refolding solubilised TCR chains in a refolding buffer comprising a solubilising agent, for example urea. Advantageously, the urea may be present at a concentration of at least 0.1M or at least 1M or at least 2.5M, or about 5M. An alternative solubilising agent which may be used is guanidine, at a concentration of between 0.1M and 8M, preferably at least 1M or at least 2.5M. Prior to refolding, a reducing agent is preferably employed to ensure complete reduction of cysteine residues. Further denaturing agents such as DTT and guanidine may be used as necessary. Different

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denaturants and reducing agents may be used prior to the refolding step (e.g. urea, β -mercaptoethanol). Alternative redox couples may be used during refolding, such as a cystamine/cysteamine redox couple, DTT or β -mercaptoethanol/atmospheric oxygen, and cysteine in reduced and oxidised forms.

5

Folding efficiency may also be increased by the addition of certain other protein components, for example chaperone proteins, to the refolding mixture. Improved refolding has been achieved by passing protein through columns with immobilised mini-chaperones (Altamirano, *et al.* (1999). *Nature Biotechnology* 17: 187-191;

10 Altamirano, *et al.* (1997). *Proc Natl Acad Sci U S A* 94(8): 3576-8).

Alternatively, soluble TCR the present invention may obtained by expression in a eukaryotic cell system, such as insect cells.

15 Purification of the TCR may be achieved by many different means. Alternative modes of ion exchange may be employed or other modes of protein purification may be used such as gel filtration chromatography or affinity chromatography.

20 Soluble TCRs and multivalent TCR complexes of the present invention also find use in screening for agents, such as small chemical compounds, which have the ability to inhibit the binding of the TCR to its pMHC complex. Thus, in a further aspect, the present invention provides a method for screening for an agent which inhibits the binding of a T cell receptor to a peptide-MHC complex, comprising monitoring the binding of a soluble T cell receptor of the invention with a peptide-MHC complex in
25 the presence of an agent; and selecting agents which inhibit such binding.

30 Suitable techniques for such a screening method include the Surface Plasmon Resonance-based method described in WO 01/22084. Other well-known techniques that could form the basis of this screening method are Scintillation Proximity Analysis (SPA) and Amplified Luminescent Proximity Assay.

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Agents selected by screening methods of the invention can be used as drugs, or as the basis of a drug development programme, being modified or otherwise improved to have characteristics making them more suitable for administration as a medicament. Such medicaments can be used for the treatment of conditions which include an
5 unwanted T cell response component. Such conditions include cancer (e.g. renal, ovarian, bowel, head & neck, testicular, lung, stomach, cervical, bladder, prostate or melanoma), autoimmune disease, graft rejection and graft versus host disease.

Preferred features of each aspect of the invention are as for each of the other aspects
10 *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

Examples

15 The invention is further described in the following examples, which do not limit the scope of the invention in any way.

Reference is made in the following to the accompanying drawings in which:

20 Figure 1 is a schematic diagram of a soluble TCR with an introduced inter-chain disulphide bond in accordance with the invention;

Figures 2a and 2b show respectively the nucleic acid sequences of the α and β chains of a soluble A6 TCR, mutated so as to introduce a cysteine codon. The shading
25 indicates the introduced cysteine codon;

Figure 3a shows the A6 TCR α chain extracellular amino acid sequence, including the $T_{48} \rightarrow C$ mutation (underlined) used to produce the novel disulphide inter-chain bond, and Figure 3b shows the A6 TCR β chain extracellular amino acid sequence, including
30 the $S_{57} \rightarrow C$ mutation (underlined) used to produce the novel disulphide inter-chain bond;

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Figure 4 is a trace obtained after anion exchange chromatography of soluble A6 TCR, showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

- 5 Figure 5 - A. Reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 4, as indicated. B. Non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 4, as indicated. Peak 1 clearly contains mainly non-disulphide linked β -chain, peak 2 contains TCR heterodimer which is inter-chain disulphide linked, and the shoulder is due to *E.coli* contaminants, mixed in with the
10 inter-chain disulphide linked sTCR, which are poorly visible on this reproduction;

Figure 6 is a trace obtained from size-exclusion chromatography of pooled fractions from peak 1 in Figure 5. The protein elutes as a single major peak, corresponding to the heterodimer;

15

Figure 7 is a BIAcore response curve of the specific binding of disulphide-linked A6 soluble TCR to HLA-A2-tax complex. Insert shows binding response compared to control for a single injection of disulphide-linked A6 soluble TCR;

- 20 Figure 8a shows the A6 TCR α chain sequence including novel cysteine residue mutated to incorporate a BamH1 restriction site. Shading indicates the mutations introduced to form the BamH1 restriction site. Figures 8b and 8c show the DNA sequence of α and β chain of the JM22 TCR mutated to include additional cysteine residues to form a non-native disulphide bond;

25

Figures 9a and 9b show respectively the JM22 TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 8a and 8b;

- Figure 10 is a trace obtained after anion exchange chromatography of soluble
30 disulphide-linked JM22 TCR showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

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Figure 11a shows a reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 10, as indicated and Figure 11b shows a non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 10, as indicated. Peak 1 clearly contains TCR heterodimer which is inter-chain disulphide linked.

5

Figure 12 is a trace obtained from size-exclusion chromatography of pooled fractions from peak 1 in figure 10. The protein elutes as a single major peak, corresponding to the heterodimer. Yield is 80%;

- 10 Figure 13 – A. BIAcore response curve of the specific binding of disulphide-linked JM22 soluble TCR to HLA-Flu complex. B. Binding response compared to control for a single injection of disulphide-linked JM22 soluble TCR;

- 15 Figures 14a and 14b show the DNA sequence of α and β chain of the NY-ESO mutated to include additional cysteine residues to form a non-native disulphide bond;

Figures 15a and 15b show respectively the NY-ESO TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 14a and 14b

- 20 Figure 16 is a trace obtained from anion exchange chromatography of soluble NY-ESO disulphide-linked TCR showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

- 25 Figure 17 – A. Reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 16, as indicated. B. Non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 16, as indicated. Peak 1 and 2 clearly contain TCR heterodimer which is inter-chain disulphide linked;

- 30 Figure 18. Size-exclusion chromatography of pooled fractions from peak 1 (A) and peak 2 (B) in figure 17. The protein elutes as a single major peak, corresponding to the heterodimer;

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Figure 19 shows a BLAcore response curve of the specific binding of disulphide-linked NY-ESO soluble TCR to HLA-NYESO complex. A. peak 1, B. peak 2;

Figures 20a and 20b show respectively the DNA sequences of the α and β chains of a soluble NY-ESO TCR, mutated so as to introduce a novel cysteine codon (indicated by shading). The sequences include the cysteine involved in the native disulphide inter-chain bond (indicated by the codon in bold);

Figures 21a and 21b show respectively the NY-ESO TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 20a and 21b;

Figure 22 shows a trace obtained from anion exchange chromatography of soluble NY-ESO TCR α^{cys} β^{cys} showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

15

Figure 23 shows a trace obtained from anion exchange chromatography of soluble NY-ESO TCR α^{cys} showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

Figure 24 shows a trace obtained from anion exchange chromatography of soluble NY-ESO TCR β^{cys} showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

Figure 25 shows a reducing SDS-PAGE (Coomassie-stained) of NY-ESO TCR α^{cys} β^{cys} , TCR α^{cys} , and TCR β^{cys} fractions from anion exchange column runs in Figures 22-24 respectively. Lanes 1 and 7 are MW markers, lane 2 is NYESOd sTCR1g4 α -cys β peak (EB/084/033); lane 3 is NYESOd sTCR1g4 α -cys β small peak (EB/084/033), lane 4 is NYESOd sTCR1g4 α β -cys (EB/084/034), lane 5 is NYESOd sTCR1g4 α -cys β -cys small peak (EB/084/035), and lane 6 is NYESOd sTCR1g4 α -cys β -cys peak (EB/084/035);

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Figure 26 shows a non-reducing SDS-PAGE (Coomassie-stained) of NY-ESO TCR α^{cys} β^{cys} , TCR α^{cys} , and TCR β^{cys} fractions from anion exchange column runs in Figures 22-24 respectively. Lanes 1 and 7 are MW markers, lane 2 is NYESOd sTCR1g4 α -cys β peak (EB/084/033); lane 3 is NYESOd sTCR1g4 α -cys β small peak (EB/084/033), lane 4 is NYESOd sTCR1g4 α β -cys (EB/084/034), lane 5 is NYESOd sTCR1g4 α -cys β -cys small peak (EB/084/035), and lane 6 is NYESOd sTCR1g4 α -cys β -cys peak (EB/084/035);

Figure 27 is a trace obtained from size exclusion exchange chromatography of soluble NY-ESO TCR α^{cys} β^{cys} showing protein elution of pooled fractions from Figure 22. The protein elutes as a single major peak, corresponding to the heterodimer;

5

Figure 28 is a trace obtained from size exclusion exchange chromatography of soluble NY-ESO TCR α^{cys} showing protein elution of pooled fractions from Figure 22. The protein elutes as a single major peak, corresponding to the heterodimer;

- 10 Figure 29 is a trace obtained from size exclusion exchange chromatography of soluble NY-ESO TCR β^{cys} showing protein elution of pooled fractions from Figure 22. The protein elutes as a single major peak, corresponding to the heterodimer;

- 15 Figure 30 is a BIAcore response curve of the specific binding of NY-ESO TCR α^{cys} β^{cys} to HLA-NY-ESO complex;

Figure 31 is a BIAcore response curve of the specific binding of NY-ESO TCR α^{cys} to HLA-NY-ESO complex;

- 20 Figure 32 is a BIAcore response curve of the specific binding of NY-ESO TCR β^{cys} to HLA-NY-ESO complex;

Figures 33a and 33b show respectively the DNA sequences of the α and β chains of a soluble AH-1.23 TCR, mutated so as to introduce a novel cysteine codon (indicated by

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shading). The sequences include the cysteine involved in the native disulphide inter-chain bond (indicated by the codon in bold);

Figures 34a and 34b show respectively the AH-1.23 TCR α and β chain extracellular
5 amino acid sequences produced from the DNA sequences of Figures 33a and 33b;

Figure 35 is a trace obtained from anion exchange chromatography of soluble AH-1.23 TCR showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

10

Figure 36 is a reducing SDS-PAGE (10% Bis-Tris gel, Coomassie-stained) of AH-1.23 TCR fractions from anion exchange column run in Figure 35. Proteins examined are the anion exchange fractions of TCR 1.23 S-S from refold 3. Lane 1 is MW markers, lane 2 is B4, lane 3 is C2, lane 4 is C3, lane 5 is C4, lane 6 is C5, lane 7 is C6, lane 8 is C7, lane 9 is C8, and lane 10 is C9;

Figure 37 is a non-reducing SDS-PAGE (10% Bis-Tris gel, Coomassie-stained) of AH-1.23 TCR fractions from anion exchange column run in Figure 35. Proteins examined are the anion exchange fractions of TCR 1.23 S-S from refold 3. Lane 1 is MW markers, lane 2 is B4, lane 3 is C2, lane 4 is C3, lane 5 is C4, lane 6 is C5, lane 7 is C6, lane 8 is C7, lane 9 is C8, and lane 10 is C9;

Figure 38 is a trace obtained from size exclusion exchange chromatography of soluble AH-1.23 TCR showing protein elution of pooled fractions from Figure 35. The
15 protein elutes as a single major peak, corresponding to the heterodimer;

Figures 39a and 39b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 48 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine
20 codon and the underlined amino acid indicates the introduced cysteine;

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Figures 40a and 40b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 45 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

5

Figures 41a and 41b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 61 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

10

Figures 42a and 42b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 50 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

15

Figures 43a and 43b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 10 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

20

Figures 44a and 44b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 15 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

25

Figures 45a and 45b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 12 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

30

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Figures 46a and 46b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 22 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the shaded amino acid indicates the introduced cysteine;

5

Figures 47a and 47b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 52 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

10

Figures 48a and 48b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 43 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

15

Figures 49a and 49b show respectively the DNA and amino acid sequences of the α chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 57 in exon 1 of TRAC*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

20

Figures 50a and 50b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 77 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

25

Figures 51a and 51b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 17 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

30

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Figures 52a and 52b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 13 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

5

Figures 53a and 53b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 59 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

10

Figures 54a and 54b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 79 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

15

Figures 55a and 55b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 14 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

20

Figures 56a and 56b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 55 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

25

Figures 57a and 57b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 63 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

30

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Figures 58a and 58b show respectively the DNA and amino acid sequences of the β chain of a soluble A6 TCR, mutated so as to introduce a novel cysteine at residue 15 in exon 1 of TRBC2*01. The shaded nucleotides indicate the introduced novel cysteine codon and the underlined amino acid indicates the introduced cysteine;

5

Figures 59-64 are traces obtained from anion exchange chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between: residues 48 of exon 1 of TRAC*01 and 57 of exon 1 of TRBC2*01; residues 45 of exon 1 of TRAC*01 and 77 of exon 1 of TRBC2*01; residues 10 of exon 1 of TRAC*01 and 17 of exon 1 of TRBC2*01; residues 45 of exon 1 of TRAC*01 and 59 of exon 1 of TRBC2*01; residues 52 of exon 1 of TRAC*01 and 55 of exon 1 of TRBC2*01; residues 15 of exon 1 of TRAC*01 and 15 of exon 1 of TRBC2*01, respectively, showing protein elution from a POROS 50 column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

15

Figures 65a and 65b are, respectively, reducing and non-reducing SDS-PAGE (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 48 of exon 1 of TRAC*01 and 57 of exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 59;

20

Figures 66a and 66b are, respectively, reducing and non-reducing SDS-PAGE (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 45 of exon 1 of TRAC*01 and 77 of exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 60;

25

Figures 67a and 67b are, respectively, reducing and non-reducing SDS-PAGE (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 10 of exon 1 of TRAC*01 and 17 exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 61;

30

Figures 68a and 68b are, respectively, reducing and non-reducing SDS-PAGE (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain

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bond between residues 45 of exon 1 of TRAC*01 and 59 of exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 62;

Figures 69a and 69b are, respectively, reducing and non-reducing SDS-PAGE
5 (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 52 of exon 1 of TRAC*01 and 55 of exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 63;

Figures 70a and 70b are, respectively, reducing and non-reducing SDS-PAGE
10 (Coomassie-stained) of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 15 of exon 1 of TRAC*01 and 15 of exon 1 of TRBC2*01, fractions run were collected from anion exchange column run in Figure 64;

Figure 71 is a trace obtained from size exclusion chromatography of soluble A6 TCR
15 containing a novel disulphide inter-chain bond between residues 48 of exon 1 of TRAC*01 and 57 of exon 1 of TRBC2*01, showing protein elution from a Superdex 200 HL gel filtration column. Fractions run were collected from anion exchange column run in Figure 59;

20 Figure 72 is a trace obtained from size exclusion chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 45 of exon 1 of TRAC*01 and 77 of exon 1 of TRBC2*01, showing protein elution from a Superdex 200 HL gel filtration column. Fractions run were collected from anion exchange column run in Figure 60;

25

Figure 73 is a trace obtained from size exclusion chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 10 of exon 1 of TRAC*01 and 17 of exon 1 of TRBC2*01, showing protein elution from a Superdex 200 HL gel filtration column. Fractions run were collected from anion exchange
30 column run in Figure 61;

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Figure 74 is a trace obtained from size exclusion chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 45 of exon 1 of TRAC*01 and 59 of exon 1 of TRBC2*01, showing protein elution from a Superdex 200 HL gel filtration column. Fractions run were collected from anion exchange
5 column run in Figure 62;

Figure 75 is a trace obtained from size exclusion chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 52 of exon 1 of TRAC*01 and 55 of exon 1 of TRBC2*01, showing protein elution from a Superdex
10 200 HL gel filtration column. Fractions run were collected from anion exchange column run in Figure 63;

Figure 76 is a trace obtained from size exclusion chromatography of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 15 of exon 1 of
15 TRAC*01 and 15 of exon 1 of TRBC2*01, showing protein elution from a Superdex 200 HL gel filtration column. Fractions run were collected from anion exchange column run in Figure 64; and

Figures 77-80 are BIAcore response curves showing, respectively, binding of soluble
20 A6 TCR containing a novel disulphide inter-chain bond between: residues 48 of exon 1 of TRAC*01 and 57 of exon 1 of TRBC2*01; residues 45 of exon 1 of TRAC*01 and 77 of exon 1 of TRBC2*01; residues 10 of exon 1 of TRAC*01 and 17 of exon 1 of TRBC2*01; and residues 45 of exon 1 of TRAC*01 and 59 of exon 1 of TRBC2*01 to HLA-A2-tax pMHC.

25

Figure 81 is a BIAcore trace showing non-specific binding of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 52 of exon 1 of TRAC*01 and 55 of exon 1 of TRBC2*01 to HLA-A2-tax and to HLA-A2-NY-ESO pMHC;

30

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Figure 82 is a BIAcore response curve showing binding of soluble A6 TCR containing a novel disulphide inter-chain bond between residues 15 of exon 1 of TRAC*01 and 15 of exon 1 of TRBC2*01 to HLA-A2-tax pMHC;

5 Figure 83a is an electron density map around the model with 1BD2 sequence (Chain A Thr164, Chain B Ser 174). Map contoured at 1.0, 2.0 and 3.0 σ . Figure 83b is an electron density map after refinement with Cys in the two positions A164 and B174. The map is contoured at the same σ levels as for Fig 83a;

10 Figure 84 compares the structures of 1BD2 TCR with an NY-ESO TCR of the present invention by overlaying said structures in ribbon and coil representations;

Figures 85a and 85b show the DNA and amino acid sequences respectively of the β chain of the NY-ESO TCR incorporating a biotin recognition site. The biotin
15 recognition site is highlighted;

Figures 86a and 86b show the DNA and amino acid sequences respectively of the β chain of the NY-ESO TCR incorporating the hexa-histidine tag. The hexa-histidine tag is highlighted;

20

Figure 87 illustrates the elution of soluble NY-ESO TCR containing a novel disulphide bond and a biotin recognition sequence from a POROS 50HQ anion exchange column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

25 Figure 88 illustrates the elution of soluble NY-ESO TCR containing a novel disulphide bond and a hexa-histidine tag from a POROS 50HQ anion exchange columns using a 0-500 mM NaCl gradient, as indicated by the dotted line;

Figure 89 is a protein elution profile from gel filtration chromatography of pooled
30 fractions from the NY-ESO-biotin tagged anion exchange column run illustrated by Figure 87;

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Figure 90 is a protein elution profile from gel filtration chromatography of pooled fractions from the NY-ESO-hexa-histidine tagged anion exchange column run illustrated by Figure 88;

- 5 Figures 91a-h are FACS histograms illustrating the staining intensity produced from 25,000 events for HLA-A2 positive EBV transformed B cell line (PP LCL) incubated with the following concentrations of NY-ESO peptide and fluorescent NY-ESO TCR tetramers respectively: NYESO 0 TCR 5 μ g, NYESO 10⁻⁴M TCR 5 μ g, NYESO 10⁻⁵M TCR 5 μ g, NYESO 10⁻⁶M TCR 5 μ g, NYESO 0 TCR 10 μ g, NYESO 10⁻⁴M TCR 10 μ g, 10 NYESO 10⁻⁵M TCR 10 μ g, NYESO 10⁻⁶M TCR 10 μ g;

Figure 92 is the DNA sequence of the beta-chain of A6 TCR incorporating the TRBC1*01 constant region;

- 15 Figure 93 is an anion exchange chromatography trace of soluble A6 TCR incorporating the TRBC1*01 constant region showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

- 20 Figure 94 – A. Reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 93, as indicated. B. Non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 93, as indicated.;

- Figure 95 – Size-exclusion chromatography of pooled fractions from peak 2 in figure 93. Peak 1 contains TCR heterodimer which is inter-chain disulphide linked; 25

Figure 96 – A. BIAcore analysis of the specific binding of disulphide-linked A6 soluble TCR to HLA-Flu complex. B.Binding response compared to control for a single injection of disulphide-linked A6 soluble TCR;

- 30 Figure 97 shows the nucleic acid sequence of the mutated beta chain of the A6 TCR incorporating the 'free' cysteine;

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Figure 98 – Anion exchange chromatography of soluble A6 TCR incorporating the ‘free’ cysteine, showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

5

Figure 99 – A. Reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 98, as indicated. B. Non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 98, as indicated;

10 Figure 100 – Size-exclusion chromatography of pooled fractions from peak 2 in figure 98. Peak 1 contains TCR heterodimer which is inter-chain disulphide linked;

Figure 101 – A. BIAcore analysis of the specific binding of disulphide-linked A6 soluble TCR incorporating the ‘free’ cysteine to HLA-Flu complex. B. Binding
15 response compared to control for a single injection of disulphide-linked A6 soluble TCR;

Figure 102 shows the nucleic acid sequence of the mutated beta chain of the A6 TCR incorporating a serine residue mutated in for the ‘free’ cysteine;

20

Figure 103 – Anion exchange chromatography of soluble A6 TCR incorporating a serine residue mutated in for the ‘free’ cysteine showing protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line;

25

Figure 104 – A. Reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 103, as indicated. B. Non-reducing SDS-PAGE (Coomassie-stained) of fractions from column run in Figure 103, as indicated. Peak 2 clearly contains TCR heterodimer which is inter-chain disulphide linked;

30

Figure 105 – Size-exclusion chromatography of pooled fractions from peak 2 in Figure 103. Peak 1 contains TCR heterodimer which is inter-chain disulphide linked;

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Figure 106 – A. BIAcore analysis of the specific binding of disulphide-linked A6 soluble TCR incorporating a serine residue mutated in for the ‘free’ cysteine to HLA-Flu complex. B. Binding response compared to control for a single injection of
5 disulphide-linked A6 soluble TCR;

Figure 107 shows the nucleotide sequence of pYX112;

Figure 108 shows the nucleotide sequence of pYX122;

10

Figure 109 shows the DNA and protein sequences of pre-pro mating factor alpha fused to TCR α chain;

Figure 110 shows the DNA and protein sequence of pre-pro mating factor alpha fused
15 to TCR β chain;

Figure 111 shows a Western Blot of soluble TCR expressed in *S. cerevisiae* strain SEY6210. Lane C contains 60ng of purified soluble NY-ESO TCR as a control. Lanes 1 and 2 contain the proteins harvested from the two separate TCR transformed
20 yeast cultures;

Figure 112 shows the nucleic acid sequence of the KpnI to EcoRI insert of the pEX172 plasmid. The remainder of the plasmid is pBlueScript II KS-;

25 Figure 113 is a schematic diagram of the TCR chains for cloning into baculovirus;

Figure 114 shows the nucleic acid sequence of disulphide A6 α TCR construct as a BamHI insert for insertion into pAcAB3 expression plasmid;

30 Figure 115 shows the disulphide A6 β TCR construct as a BamHI for insertion into pAcAB3 expression plasmid; and

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Figure 116 shows a Coomassie stained gel and Western Blot against the bacterially-produced disulphide A6 TCR and the Insect disulphide A6 TCR.

In all of the following examples, unless otherwise stated, the soluble TCR chains
5 produced are truncated immediately C-terminal to the cysteine residues which form the native interchain disulphide bond.

Example 1 – Design of primers and mutagenesis of A6 Tax TCR α and β chains

10 For mutating A6 Tax threonine 48 of exon 1 in TRAC*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C ACA GAC AAA tgT GTG CTA GAC AT

5'-AT GTC TAG CAC Aca TTT GTC TGT G

15

For mutating A6 Tax serine 57 of exon 1 in both TRBC1*01 and TRBC2*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C AGT GGG GTC tGC ACA GAC CC

20 5'-GG GTC TGT GCa GAC CCC ACT G

PCR mutagenesis:

Expression plasmids containing the genes for the A6 Tax TCR α or β chain were mutated using the α -chain primers or the β -chain primers respectively, as follows.

25 100 ng of plasmid was mixed with 5 μ l 10 mM dNTP, 25 μ l 10xPfu-buffer (Stratagene), 10 units Pfu polymerase (Stratagene) and the final volume was adjusted to 240 μ l with H₂O. 48 μ l of this mix was supplemented with primers diluted to give a final concentration of 0.2 μ M in 50 μ l final reaction volume. After an initial
denaturation step of 30 seconds at 95°C, the reaction mixture was subjected to 15
30 rounds of denaturation (95°C, 30 sec.), annealing (55°C, 60 sec.), and elongation (73°C, 8 min.) in a Hybaid PCR express PCR machine. The product was then digested for 5 hours at 37°C with 10 units of DpnI restriction enzyme (New England Biolabs).

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10 μ l of the digested reaction was transformed into competent XL1-Blue bacteria and grown for 18 hours at 37°C. A single colony was picked and grown over night in 5 ml TYP + ampicillin (16 g/l Bacto-Tryptone, 16 g/l Yeast Extract, 5 g/l NaCl, 2.5 g/l K₂HPO₄, 100 mg/l Ampicillin). Plasmid DNA was purified on a Qiagen mini-prep
 5 column according to the manufacturer's instructions and the sequence was verified by automated sequencing at the sequencing facility of Department of Biochemistry, Oxford University. The respective mutated nucleic acid and amino acid sequences are shown in Figures 2a and 3a for the α chain and Figures 2b and 3b for the β chain.

10 *Example 2 – Expression, refolding and purification of soluble TCR*

The expression plasmids containing the mutated α -chain and β -chain respectively were transformed separately into *E.coli* strain BL21pLysS, and single ampicillin-resistant colonies were grown at 37°C in TYP (ampicillin 100 μ g/ml) medium to OD₆₀₀
 15 of 0.4 before inducing protein expression with 0.5mM IPTG. Cells were harvested three hours post-induction by centrifugation for 30 minutes at 4000rpm in a Beckman J-6B. Cell pellets were re-suspended in a buffer containing 50mM Tris-HCl, 25% (w/v) sucrose, 1mM NaEDTA, 0.1% (w/v) NaAzide, 10mM DTT, pH 8.0. After an overnight freeze-thaw step, re-suspended cells were sonicated in 1 minute bursts for a
 20 total of around 10 minutes in a Milsonix XL2020 sonicator using a standard 12mm diameter probe. Inclusion body pellets were recovered by centrifugation for 30 minutes at 13000rpm in a Beckman J2-21 centrifuge. Three detergent washes were then carried out to remove cell debris and membrane components. Each time the inclusion body pellet was homogenised in a Triton buffer (50mM Tris-HCl, 0.5%
 25 Triton X100, 200mM NaCl, 10mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT, pH 8.0) before being pelleted by centrifugation for 15 minutes at 13000rpm in a Beckman J2-21. Detergent and salt was then removed by a similar wash in the following buffer: 50mM Tris-HCl, 1mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT, pH 8.0. Finally, the inclusion bodies were divided into 30 mg aliquots and frozen at -70°C. Inclusion
 30 body protein yield was quantitated by solubilising with 6M guanidine-HCl and measurement with a Bradford dye-binding assay (PerBio).

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Approximately 30mg (i.e. 1 μ mole) of each solubilised inclusion body chain was thawed from frozen stocks, samples were then mixed and the mixture diluted into 15ml of a guanidine solution (6 M Guanidine-hydrochloride, 10mM Sodium Acetate, 10mM EDTA), to ensure complete chain de-naturation. The guanidine solution

5 containing fully reduced and denatured TCR chains was then injected into 1 litre of the following refolding buffer: 100mM Tris pH 8.5, 400mM L-Arginine, 2mM EDTA, 5mM reduced Glutathione, 0.5mM oxidised Glutathione, 5M urea, 0.2mM PMSF. The solution was left for 24 hrs. The refold was then dialysed twice, firstly against 10 litres of 100mM urea, secondly against 10 litres of 100mM urea, 10mM Tris pH 8.0.

10 Both refolding and dialysis steps were carried out at 6-8°C.

sTCR was separated from degradation products and impurities by loading the dialysed refold onto a POROS 50HQ anion exchange column and eluting bound protein with a gradient of 0-500mM NaCl over 50 column volumes using an Akta purifier

15 (Pharmacia) as in Figure 4. Peak fractions were stored at 4°C and analysed by Coomassie-stained SDS-PAGE (Figure 5) before being pooled and concentrated. Finally, the sTCR was purified and characterised using a Superdex 200HR gel filtration column (Figure 6) pre-equilibrated in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a

20 relative molecular weight of approximately 50 kDa was pooled and concentrated prior to characterisation by BIAcore surface plasmon resonance analysis.

Example 3 – BIAcore surface plasmon resonance characterisation of sTCR binding to specific pMHC

25

A surface plasmon resonance biosensor (BIAcore 3000™) was used to analyse the binding of a sTCR to its peptide-MHC ligand. This was facilitated by producing single pMHC complexes (described below) which were immobilised to a streptavidin-coated binding surface in a semi-oriented fashion, allowing efficient testing of the

30 binding of a soluble T-cell receptor to up to four different pMHC (immobilised on separate flow cells) simultaneously. Manual injection of HLA complex allows the precise level of immobilised class I molecules to be manipulated easily.

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Such immobilised complexes are capable of binding both T-cell receptors and the coreceptor CD8 $\alpha\alpha$, both of which may be injected in the soluble phase. Specific binding of TCR is obtained even at low concentrations (at least 40 μ g/ml), implying the TCR is relatively stable. The pMHC binding properties of sTCR are observed to be qualitatively and quantitatively similar if sTCR is used either in the soluble or immobilised phase. This is an important control for partial activity of soluble species and also suggests that biotinylated pMHC complexes are biologically as active as non-biotinylated complexes.

10 Biotinylated class I HLA-A2 - peptide complexes were refolded *in vitro* from bacterially-expressed inclusion bodies containing the constituent subunit proteins and synthetic peptide, followed by purification and *in vitro* enzymatic biotinylation (O'Callaghan *et al.* (1999) *Anal. Biochem.* 266: 9-15). HLA-heavy chain was expressed with a C-terminal biotinylation tag which replaces the transmembrane and cytoplasmic domains of the protein in an appropriate construct. Inclusion body expression levels of ~75 mg/litre bacterial culture were obtained. The HLA light-chain or β 2-microglobulin was also expressed as inclusion bodies in *E.coli* from an appropriate construct, at a level of ~500 mg/litre bacterial culture.

20 *E. coli* cells were lysed and inclusion bodies are purified to approximately 80% purity. Protein from inclusion bodies was denatured in 6 M guanidine-HCl, 50 mM Tris pH 8.1, 100 mM NaCl, 10 mM DTT, 10 mM EDTA, and was refolded at a concentration of 30 mg/litre heavy chain, 30 mg/litre β 2m into 0.4 M L-Arginine-HCl, 100 mM Tris pH 8.1, 3.7mM cystamine, 6.6mM β -cysteamine, 4 mg/ml peptide (e.g. tax 11-19), by addition of a single pulse of denatured protein into refold buffer at < 5°C. Refolding was allowed to reach completion at 4°C for at least 1 hour.

30 Buffer was exchanged by dialysis in 10 volumes of 10 mM Tris pH 8.1. Two changes of buffer were necessary to reduce the ionic strength of the solution sufficiently. The protein solution was then filtered through a 1.5 μ m cellulose acetate filter and loaded onto a POROS 50HQ anion exchange column (8 ml bed volume). Protein was eluted with a linear 0-500 mM NaCl gradient. HLA-A2-peptide complex eluted at

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approximately 250 mM NaCl, and peak fractions were collected, a cocktail of protease inhibitors (Calbiochem) was added and the fractions were chilled on ice.

5 Biotinylation tagged HLA complexes were buffer exchanged into 10 mM Tris pH 8.1, 5 mM NaCl using a Pharmacia fast desalting column equilibrated in the same buffer. Immediately upon elution, the protein-containing fractions were chilled on ice and protease inhibitor cocktail (Calbiochem) was added. Biotinylation reagents were then added: 1 mM biotin, 5 mM ATP (buffered to pH 8), 7.5 mM MgCl₂, and 5 µg/ml BirA enzyme (purified according to O'Callaghan *et al.* (1999) *Anal. Biochem.* 266: 9-10 15). The mixture was then allowed to incubate at room temperature overnight.

Biotinylated HLA complexes were purified using gel filtration chromatography. A Pharmacia Superdex 75 HR 10/30 column was pre-equilibrated with filtered PBS and 1 ml of the biotinylation reaction mixture was loaded and the column was developed 15 with PBS at 0.5 ml/min. Biotinylated HLA complexes eluted as a single peak at approximately 15 ml. Fractions containing protein were pooled, chilled on ice, and protease inhibitor cocktail was added. Protein concentration was determined using a Coomassie-binding assay (PerBio) and aliquots of biotinylated HLA complexes were stored frozen at -20°C. Streptavidin was immobilised by standard amine coupling 20 methods.

The interactions between A6 Tax sTCR containing a novel inter-chain bond and its ligand/ MHC complex or an irrelevant HLA-peptide combination, the production of which is described above, were analysed on a BLAcore 3000™ surface plasmon 25 resonance (SPR) biosensor. SPR measures changes in refractive index expressed in response units (RU) near a sensor surface within a small flow cell, a principle that can be used to detect receptor ligand interactions and to analyse their affinity and kinetic parameters. The probe flow cells were prepared by immobilising the individual HLA-peptide complexes in separate flow cells via binding between the biotin cross linked 30 onto β2m and streptavidin which have been chemically cross linked to the activated surface of the flow cells. The assay was then performed by passing sTCR over the surfaces of the different flow cells at a constant flow rate, measuring the SPR response

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in doing so. Initially, the specificity of the interaction was verified by passing sTCR at a constant flow rate of 5 $\mu\text{l min}^{-1}$ over two different surfaces; one coated with ~ 5000 RU of specific peptide-HLA complex, the second coated with ~ 5000 RU of non-specific peptide-HLA complex (Figure 7 insert). Injections of soluble sTCR at constant flow rate and different concentrations over the peptide-HLA complex were used to define the background resonance. The values of these control measurements were subtracted from the values obtained with specific peptide-HLA complex and used to calculate binding affinities expressed as the dissociation constant, K_d (Price & Dwek, Principles and Problems in Physical Chemistry for Biochemists (2nd Edition) 1979, Clarendon Press, Oxford), as in Figure 7.

The K_d value obtained ($1.8\mu\text{M}$) is close to that reported for the interaction between A6 Tax sTCR without the novel di-sulphide bond and pMHC ($0.91\mu\text{M}$ – Ding *et al*, 1999, *Immunity* 11:45-56).

15

Example 4 – Production of soluble JM22 TCR containing a novel disulphide bond.

The β chain of the soluble A6 TCR prepared in Example 1 contains in the native sequence a BglII restriction site (AAGCTT) suitable for use as a ligation site.

20

PCR mutagenesis was carried as detailed below to introduce a BamHI restriction site (GGATCC) into the α chain of soluble A6 TCR, 5' of the novel cysteine codon. The sequence described in Figure 2a was used as a template for this mutagenesis. The following primers were used:

25

| BamHI |

5' -ATATCCAGAACCCgGA $\text{\textcircled{C}}$ CCTGCCGTGTA-3'

5' -TACACGGCAGGAaTCcGGGTTCTGGATAT-3'

30

100 ng of plasmid was mixed with 5 μl 10 mM dNTP, 25 μl 10xPfu-buffer (Stratagene), 10 units Pfu polymerase (Stratagene) and the final volume was adjusted to 240 μl with H_2O . 48 μl of this mix was supplemented with primers diluted to give a

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final concentration of 0.2 μ M in 50 μ l final reaction volume. After an initial denaturation step of 30 seconds at 95°C, the reaction mixture was subjected to 15 rounds of denaturation (95°C, 30 sec.), annealing (55°C, 60 sec.), and elongation (73°C, 8 min.) in a Hybaid PCR express PCR machine. The product was then digested for 5 hours at 37°C with 10 units of DpnI restriction enzyme (New England Biolabs). 10 μ l of the digested reaction was transformed into competent XL1-Blue bacteria and grown for 18 hours at 37°C. A single colony was picked and grown over night in 5 ml TYP + ampicillin (16 g/l Bacto-Tryptone, 16 g/l Yeast Extract, 5 g/l NaCl, 2.5 g/l K₂HPO₄, 100 mg/l Ampicillin). Plasmid DNA was purified on a Qiagen mini-prep column according to the manufacturer's instructions and the sequence was verified by automated sequencing at the sequencing facility of Department of Biochemistry, Oxford University. The mutations introduced into the α chain were "silent", therefore the amino acid sequence of this chain remained unchanged from that detailed in Figure 3a. The DNA sequence for the mutated α chain is shown in Figure 8a.

15

In order to produce a soluble JM22 TCR incorporating a novel disulphide bond, A6 TCR plasmids containing the α chain BamHI and β chain BglII restriction sites were used as templates. The following primers were used:

20

| NdeI |

5' -GGAGATATACATATGCAACTACTAGAACAA-3'

5' -TACACGGCAGGATCCGGGTCTGGATATT-3'

| BamHI |

25

| NdeI |

5' -GGAGATATACATATGGTGGATGGTGGGAATC-3'

5' -CCCAAGCTTAGTCTGCTCTACCCCAGGCCTCGGC-3'

| BglII |

30

JM22 TCR α and β -chain constructs were obtained by PCR cloning as follows. PCR reactions were performed using the primers as shown above, and templates containing the JM22 TCR chains. The PCR products were restriction digested with

-46-

the relevant restriction enzymes, and cloned into pGMT7 to obtain expression plasmids. The sequence of the plasmid inserts were confirmed by automated DNA sequencing. Figures 8b and 8c show the DNA sequence of the mutated α and β chains of the JM22 TCR respectively, and Figures 9a and 9b show the resulting amino acid sequences.

The respective TCR chains were expressed, co-refolded and purified as described in Examples 1 and 2. Figure 10 illustrates the elution of soluble disulphide-linked JM22 TCR protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figure 11 shows the results of both reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels of fractions from the column run illustrated by Figure 10. Peak 1 clearly contains TCR heterodimer which is inter-chain disulphide linked. Figure 12 shows protein elution from a size-exclusion column of pooled fractions from peak 1 in Figure 10.

A BIAcore analysis of the binding of the JM22 TCR to pMHC was carried out as described in Example 3. Figure 13a shows BIAcore analysis of the specific binding of disulphide-linked JM22 soluble TCR to HLA-Flu complex. Figure 13b shows the binding response compared to control for a single injection of disulphide-linked JM22 soluble TCR. The K_d of this disulphide-linked TCR for the HLA-flu complex was determined to be $7.9 \pm 0.51 \mu\text{M}$

Example 5 – Production of soluble NY-ESO TCR containing a novel disulphide bond

cDNA encoding NY-ESO TCR was isolated from T cells supplied by Enzo Cerundolo (Institute of Molecular Medicine, University of Oxford) according to known techniques. cDNA encoding NY-ESO TCR was produced by treatment of the mRNA with reverse transcriptase.

In order to produce a soluble NY-ESO TCR incorporating a novel disulphide bond, A6 TCR plasmids containing the α chain BamHI and β chain BglII restriction sites were used as templates as described in Example 4. The following primers were used:

25 *Denaturation of soluble TCRs*; 30mg of the solubilised TCR β -chain inclusion body and 60mg of the solubilised TCR α -chain inclusion body was thawed from frozen stocks. The inclusion bodies were diluted to a final concentration of 5mg/ml in 6M guanidine solution, and DTT (2M stock) was added to a final concentration of 10mM. The mixture was incubated at 37°C for 30 min.

30 *Refolding of soluble TCRs*: 1 L refolding buffer was stirred vigorously at 5°C $\pm$ 3°C. The redox couple (2-mercaptoethylamine and cystamine (to final concentrations of 6.6mM and 3.7mM, respectively) were added approximately 5 minutes before addition

-48-

of the denatured TCR chains. The protein was then allowed to refold for approximately 5 hours $\pm$ 15 minutes with stirring at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Dialysis of refolded soluble TCRs: The refolded TCR was dialysed in Spectrapor 1 membrane (Spectrum; Product No. 132670) against 10 L 10 mM Tris pH 8.1 at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 18-20 hours. After this time, the dialysis buffer was changed to fresh 10 mM Tris pH 8.1 (10 L) and dialysis was continued at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for another 20-22 hours.

Figure 16 illustrates the elution of soluble NY-ESO disulphide-linked TCR protein elution from a POROS 50HQ column using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figure 17 shows the results of both reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels of fractions from the column run illustrated by Figure 16. Peaks 1 and 2 clearly contain TCR heterodimer which is inter-chain disulphide linked. Figure 18 shows size-exclusion chromatography of pooled fractions from peak 1 (A) and peak 2 (B) in Figure 17. The protein elutes as a single major peak, corresponding to the heterodimer.

A BIAcore analysis of the binding of the disulphide-linked NY-ESO TCR to pMHC was carried out as described in Example 3. Figure 19 shows BIAcore analysis of the specific binding of disulphide-linked NY-ESO soluble TCR to HLA-NYESO complex. A. peak 1, B. peak 2.

The K_d of this disulphide-linked TCR for the HLA-NY-ESO complex was determined to be $9.4 \pm 0.84 \mu\text{M}$.

Example 6 – Production of soluble NY-ESO TCR containing a novel disulphide inter-chain bond, and at least one of the two cysteines required to form the native disulphide inter-chain bond

In order to produce a soluble NY-ESO TCR incorporating a novel disulphide bond and at least one of the cysteine residues involved in the native disulphide inter-chain bond,

-49-

plasmids containing the α chain BamHI and β chain BglII restriction sites were used as a framework as described in Example 4. The following primers were used:

- | NdeI |
- 5 5' -GGAGATATACATATGCAGGAGGTGACACAG-3'
- | HindIII |
- 5' -CCCAAGCTTAACAGGAACTTTCTGGGCTGGGGAAGAA-3'
- | NdeI |
- 10 5' -GGAGATATACATATGGGTGTCACTCAGACC-3'
- 5' -CCCAAGCTTAACAGTCTGCTCTACCCAGGCCTCGGC -3'
- | BglII |
- 15 NY-ESO TCR α and β -chain constructs were obtained by PCR cloning as follows. PCR reactions were performed using the primers as shown above, and templates containing the NY-ESO TCR chains. The PCR products were restriction digested with the relevant restriction enzymes, and cloned into pGMT7 to obtain expression plasmids. The sequence of the plasmid inserts were confirmed by automated DNA
- 20 sequencing. Figures 20a and 20b show the DNA sequence of the mutated α and β chains of the NY-ESO TCR respectively, and Figures 21a and 21b show the resulting amino acid sequences.
- To produce a soluble NY-ESO TCR containing both a non-native disulphide inter-
- 25 chain bond and the native disulphide inter-chain bond, DNA isolated using both of the above primers was used. To produce soluble NY-ESO TCRs with a non-native disulphide inter-chain bond and only one of the cysteine residues involved in the native disulphide inter-chain bond, DNA isolated using one of the above primers together with the appropriate primer from Example 5 was used.
- 30
- The respective TCR chains were expressed, co-refolded and purified as described in Example 5.

-50-

Figures 22-24 illustrate the elution of soluble NY-ESO TCR α^{cys} β^{cys} (i.e. with non-native and native cysteines in both chains), TCR α^{cys} (with non-native cysteines in both chains but the native cysteine in the α chain only), and TCR β^{cys} (with non-native cysteines in both chains but the native cysteine in the β chain only) protein elution from POROS 50HQ anion exchange columns using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figures 25 and 26 respectively show the results of reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels of fractions from the NY-ESO TCR α^{cys} β^{cys} , TCR α^{cys} , and TCR β^{cys} column runs illustrated by Figures 22-24. These clearly indicate that TCR heterodimers which are inter-chain disulphide linked have been formed. Figures 27-29 are protein elution profiles from gel filtration chromatography of pooled fractions from the NY-ESO TCR α^{cys} β^{cys} , TCR α^{cys} , and TCR β^{cys} anion exchange column runs illustrated by Figures 22-24 respectively. The protein elutes as a single major peak, corresponding to the TCR heterodimer.

A BIAcore analysis of sTCR binding to pMHC was carried out as described in Example 3. Figures 30-32 show BIAcore analysis of the specific binding of NY-ESO TCR α^{cys} β^{cys} , TCR α^{cys} , and TCR β^{cys} respectively to HLA-NYESO complex.

TCR α^{cys} β^{cys} had a K_d of $18.08 \pm 2.075 \mu\text{M}$, TCR α^{cys} had a K_d of $19.24 \pm 2.01 \mu\text{M}$, and TCR β^{cys} had a K_d of $22.5 \pm 4.0692 \mu\text{M}$.

Example 7 – Production of soluble AH-1.23 TCR containing a novel disulphide inter-chain bond

cDNA encoding AH-1.23 TCR was isolated from T cells supplied by Hill Gaston (Medical School, Addenbrooke's Hospital, Cambridge) according to known techniques. cDNA encoding NY-ESO TCR was produced by treatment of the mRNA with reverse transcriptase.

In order to produce a soluble AH-1.23 TCR incorporating a novel disulphide bond, TCR plasmids containing the α chain BamHI and β chain BglII restriction sites were used as a framework as described in Example 4. The following primers were used:

Figure 35 illustrates the elution of soluble AH-1.23 disulphide-linked TCR protein elution from a POROS 50HQ anion exchange column using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figures 36 and 37 show the results of reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels respectively of fractions from the column run illustrated by Figure 35. These gels clearly indicate the presence of a TCR heterodimer which is inter-chain disulphide linked. Figure 38 is the elution profile from a Superdex 75 HR gel filtration column of pooled fractions from the anion exchange column run illustrated in

-52-

Figure 35. The protein elutes as a single major peak, corresponding to the heterodimer.

Example 8 – Production of soluble A6 TCRs containing a novel disulphide inter-chain bond at alternative positions within the immunoglobulin region of the constant domain

The following experiments were carried out in order to investigate whether it was possible to form functional soluble TCRs which include a novel disulphide bond in the TCR immunoglobulin region at a position other than between threonine 48 of exon 1 in TRAC*01 and serine 57 of exon 1 in both TRBC1*01 / TRBC2*01.

For the mutating the A6 TCR α -chain, the following primers were designed (the numbers in the primer names refer to the position of the amino acid residue to be mutated in exon 1 of TRAC*01, mutated residues are shown in lower case):

15

T48→C Mutation

5'-CACAGACAAAtgTGTGCTAGACAT-3'

5'-ATGTCTAGCACAcTTTGTCTGTG-3'

20 Y10→C Mutation

5'-CCCTGCCGTGTgCCAGCTGAGAG-3''

5'-CTCTCAGCTGGcACACGGCAGGG-3'

L12→C Mutation

25 5'-CCGTGTACCAGtgcAGAGACTCTAAATC-3'

5'-GATTAGAGTCTCTgcaCTGGTACACGG-3'

S15→C Mutation

5'-CAGCTGAGAGACTgTAAATCCAGTGAC-3'

30 5'-GTCACTGGATTTAcAGTCTCTCAGCTG-3'

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V22→C Mutation

5'-CAGTGACAAGTCTtgCTGCCTATTCAC-3'

5'-GTGAATAGGCAGcaAGACTTGTCAC-3'

5 Y43→C Mutation

5'-GATTCTGATGTGTgTATCACAGACAAAT-3'

5'-ATTTGTCTGTGATAcACACATCAGAATC-3'

T45→C Mutation

10 5'-CTGATGTGTATATCtgtGACAAAAC-3'

5'-GCACAGTTTTGTCacaGATATACACATCAG-3'

L50→C Mutation

5'-AGACAAAAC-3'

15 5'-AGACCTCATGTCacaCACAGTTTTGTCT-3'

M52→C Mutation

5'-ACTGTGCTAGACtgtAGGTCTATGGAC-3'

5'-GTCCATAGACCTacaGTCTAGCACAGT-3'

20

S61→C Mutation

5'-CTTCAAGAGCAACtGTGCTGTGGCC-3'

5'-GGCCACAGCACaGTTGCTCTTGAAG-3'

- 25 For mutating the TCR A6 β -chain, the following primers were designed (the numbers in the primer names refer to the position of the amino acid residue to be mutated in exon 1 of TRBC2*01. Mutated residues are shown in lower case):

S57→C Mutation

30 5'-CAGTGGGGTcGCACAGACCC-3'

5'-GGGTCTGTGCaGACCCCACTG-3'

-54-

V13→C Mutation

5'-CCGAGGTCGCTtgtTTTGAGCCATCAG-3'

5'-CTGATGGCTCAAAacaAGCGACCTCGG-3'

5

F14→C Mutation

5'-GGTCGCTGTGtgtGAGCCATCAGA-3'

5'-TCTGATGGCTCacaCACAGCGACC-3'

10 S17→C Mutation

5'-GTGTTTGAGCCATgtGAAGCAGAGATC-3'

5'-GATCTCTGCTTCacATGGCTCAAACAC-3'

G55→C Mutation

15 5'-GAGGTGCACAGTtGtGTCAGCACAGAC-3'

5'-GTCTGTGCTGACaCaACTGTGCACCTC-3'

D59→C Mutation

5'-GGGTCAGCACAtgCCCGCAGCCC-3'

20 5'-GGGCTGCGGGGcaTGTGCTGACCC-3'

L63→C Mutation

5'-CCCGCAGCCctgCAAGGAGCAGC-3'

5'-GCTGCTCCTTGCaGGGCTGCGGG-3'

25

S77→C Mutation

5'-AGATACGCTCTGtGCAGCCGCCT-3'

5'-AGGCGGCTGCaCAGAGCGTATCT-3'

30 R79→C Mutation

5'-CTCTGAGCAGctGCCTGAGGGTC-3'

-55-

5'-GACCCTCAGGCaGCTGCTCAGAG-3'

E15→C Mutation

5' -GCTGTGTTTtgtCCATCAGAA- 3'

5 5' -TTCTGATGGacaAAACACAGC- 3'

PCR mutagenesis, α and β TCR construct amplification, ligation and plasmid purification was carried out as described in Example 1 using the appropriate combination of the above primers in order to produce soluble TCRs including novel

10 disulphide inter-chain bonds between the following pairs of amino acids:

TCR α chain	TCR β chain	α Primer used	β Primer used
Thr 48	Ser 57	T48→C	S57→C
Thr 45	Ser 77	T45→C	S77→C
Ser 61	Ser 57	S61→C	S57→C
Leu 50	Ser 57	L50→C	S57→C
Tyr 10	Ser 17	Y10→C	S17→C
Ser 15	Val 13	S15→C	V13→C
Thr 45	Asp 59	T45→C	D59→C
Leu 12	Ser 17	L12→C	S17→C
Ser 61	Arg 79	S61→C	R79→C
Leu 12	Phe 14	L12→C	F14→C
Val 22	Phe 14	V22→C	F14→C
Met 52	Gly 55	M52→C	G55→C
Tyr 43	Leu 63	Y43→C	L63→C
Ser 15	Glu 15	S15→C	E15→C

Figures 39 to 58 show the DNA and amino acid sequences of the mutated A6 TCR chains amplified by the above primers. The codons encoding the mutated cysteines

15 are highlighted.

-56-

The respective TCR chains were expressed, co-refolded and purified as described in Example 5. Following purification on POROS 50HQ anion exchange column, the resulting proteins were run on SDS-Page gels in order to assess whether any correctly-refolded soluble TCR had been formed. These gels were also assessed to ascertain the presence or absence of any disulphide-linked protein of the correct molecular weight in the purified material. TCRs under investigation containing the following novel disulphide inter-chain bonds failed to produce disulphide-linked protein of the correct molecular weight using this bacterial expression system and these were not further assessed. However, alternative prokaryotic or eukaryotic expression systems are available.

TCR α chain	TCR β chain
Ser 61	Ser 57
Leu 50	Ser 57
Ser 15	Val 13
Leu 12	Ser 17
Ser 61	Arg 79
Leu 12	Phe 14
Val 22	Phe 14
Tyr 43	Leu 63

Figures 59 to 64 respectively illustrate the elution of soluble TCRs containing novel disulphide interchain bonds between the following residues: Thr 48-Ser 57, Thr 45-Ser 77, Tyr 10-Ser 17, Thr 45-Asp 59, Met 52-Gly 55 and Ser 15-Glu 15 from a POROS 200HQ anion exchange column using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figures 65 to 70 show the results of reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels respectively of fractions from the column runs illustrated by Figures 59 to 64. These gels clearly indicate the presence of TCR heterodimers that are inter-chain disulphide linked.

Figures 71 to 76 are elution profiles from a Superdex 200 HR gel filtration column of pooled fractions from the anion exchange column runs illustrated in Figures 59 to 64.

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A BIAcore analysis of the binding of the TCRs to pMHC was carried out as described in Example 3. Figures 77- 82 are BIAcore traces demonstrating the ability of the purified soluble TCRs to bind to HLA-A2 tax pMHC complexes.

- 5 Thr 48-Ser 57 had a K_d of 7.8 μ M, Thr 45-Ser 77 had a K_d of 12.7 μ M, Tyr 10-Ser 17 had a K_d of 34 μ M, Thr 45-Asp 59 had a K_d of 14.9 μ M, and Ser 15-Glu 15 had a K_d of 6.3 μ M. Met 52-Gly 55 was capable of binding to its native "target", the HLA-A2 tax complex, although it also bound in a similar manner to an "irrelevant" target, the HLA-A2-NY-ESO complex (see Figure 81)

10

Example 9 – X-ray crystallography of the disulphide-linked NY-ESO T cell receptor, specific for the NY-ESO-HLA-A2 complex.

The NY-ESO dsTCR was cloned as described in Example 5, and expressed as follows.

15

- The expression plasmids containing the mutated α -chain and β -chain respectively were transformed separately into *E.coli* strain BL21 pLysS, and single ampicillin-resistant colonies were grown at 37°C in TYP (ampicillin 100 μ g/ml) medium to OD₆₀₀ of 0.7 before inducing protein expression with 0.5mM IPTG. Cells were harvested 18 hours post-induction by centrifugation for 30 minutes at 4000rpm in a Beckman J-6B. Cell pellets were resuspended in lysis buffer containing 10mM Tris-HCl pH 8.1, 10 mM MgCl₂, 150 mM NaCl, 2 mM DTT, 10% glycerol. For every 1 L of bacterial culture 100 μ l of lysozyme (20 mg/ml) and 100 μ l of Dnase I (20 μ g/ml) were added. After incubation on ice for 30 minutes, the bacterial suspension was sonicated in 1 minute bursts for a total of 10 minutes using a Milsonix XL2020 sonicator with a standard 12mm diameter probe. Inclusion body pellets were recovered by centrifugation for 30 minutes at 13000rpm in a Beckman J2-21 centrifuge (4 °C). Three washes were then carried out in Triton wash buffer (50mM Tris-HCl pH 8.1, 0.5% Triton-X100, 100mM NaCl, 10mM NaEDTA, 0.1% (w/v), 2mM DTT) to remove cell debris and membrane components. Each time, the inclusion body pellet was homogenised in Triton wash buffer before being pelleted by centrifugation for 15 minutes at 13000rpm in a Beckman J2-21. Detergent and salt was then removed by a

25
30

-58-

similar wash in Resuspension buffer (50mM Tris-HCl pH 8.1 100mM NaCl, 10mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT). Finally, the inclusion bodies were solubilised in 6 M guanidine buffer (6 M Guanidine-hydrochloride, 50mM Tris pH 8.1, 100mM NaCl, 10mM EDTA, 10mM DTT), divided into 120 mg aliquots and
5 frozen at -70°C. Inclusion bodies were quantitated by solubilising with 6M guanidine-HCl and measurement with a Bradford dye-binding assay (PerBio).

Approximately 60mg (i.e. 2.4 μ mole) of frozen solubilised alpha chain was mixed with 30 mg (i.e. 1.2 μ mole) of frozen solubilised beta chain. The TCR mixture was
10 diluted to a final volume of 18ml with 6 M guanidine buffer and heated to 37 °C for 30 min to ensure complete chain denaturation. The guanidine solution containing fully reduced and denatured TCR chains was then mixed into 1 litre of cold refolding buffer (100mM Tris pH 8.1, 400mM L-Arginine-HCl, 2mM EDTA, 6.6 mM 2-mercapthoethylamine, 3.7 mM Cystamine, 5M urea) with stirring. The solution was
15 left for 5 hrs in the cold room (5°C $\pm$ 3°C) to allow refolding to take place. The refold was then dialysed against 12 litres of water for 18-20 hours, followed by 12 litres of 10mM Tris pH 8.1 for 18-20 hours (5°C $\pm$ 3°C). Spectrapor 1 (Spectrum Laboratories product no. 132670) dialysis membrane that has a molecular weight cut off of 6-8000kDa was used for this dialysis process. The dialysed protein was filtered through
20 0.45 μ m pore size filters (Schleicher and Schuell, Ref. number, 10 404012) fitted to a Nalgene filtration unit.

The refolded NY-ESO TCR was separated from degradation products and impurities by loading the dialysed refold onto a POROS 50HQ (Applied Biosystems) anion
25 exchange column using an ÄKTA purifier (Amersham Biotech). A POROS 50 HQ column was pre-equilibrated with 10 column volumes of buffer A (10 mM Tris pH 8.1) prior to loading with protein. The bound protein was eluted with a gradient of 0-500mM NaCl over 7 column volumes. Peak fractions (1 ml) were analysed on denaturing SDS-PAGE using reducing and non-reducing sample buffer. Peak
30 fractions containing the heterodimeric alpha-beta complex were further purified using a Superdex 75HR gel filtration column pre-equilibrated in 25 mM MES pH 6.5. The protein peak eluting at a relative molecular weight of approximately 50 kDa was

-59-

pooled, concentrated to 42 mg/ml in Ultrafree centrifugal concentrators (Millipore, part number UFV2BGC40) and stored at -80°C .

Crystallisation of NY-ESO TCR was performed by hanging drop technique at 18°C using 1 μl of protein solution (8.4 mg/ml) in 5 mM Mes pH 6.5 mixed with an equivalent volume of crystallisation buffer. Crystals appeared under several different conditions using Crystal Screen buffers (Hampton Research). Single cubic crystals ($< 100\text{ }\mu\text{m}$) were grown in 30 % PEG 4000, 0.1 M Na Citrate pH 5.6, 0.2 M ammonium acetate buffer and used for structure determination.

10

Crystals of the NY-ESO TCR were flash-frozen and tested for diffraction in the X-ray beam of the Daresbury synchrotron. The crystals diffracted to 0.25nm ($2.5\text{\AA}$) resolution. One data set was collected and processed to give a 98.6% complete set of amplitudes that were reasonable to around 0.27 nm ($2.7\text{\AA}$), but usable up to 0.25 nm ($2.5\text{\AA}$). The merging R-factor, i.e. the agreement between multiple measurements of crystallographically equivalent reflections, was 10.8% for all the data. This is marginal at the highest resolution shell. The space group was $P2_1$, with cell dimensions $a=4.25\text{ nm}$ ($42.5\text{\AA}$), $b=5.95\text{ nm}$ ($59.5\text{\AA}$), $c=8.17\text{ nm}$ ($81.7\text{\AA}$), $\beta=91.5^{\circ}$. The cell dimensions and symmetry meant there were two copies in the cell. The asymmetric unit, au or the minimum volume that needs to be studied, has only 1 molecule, and the other molecule in the cell is generated by the 2_1 symmetry operation. The positioning of the molecule in the au is arbitrary in the y-direction. As long as it is in the correct position in the x-z plane, it can be translated at will in the y-direction. This is referred to as a free parameter, in this 'polar' space group.

25

The PDB data base has only one entry containing an A/B heterodimeric TCR, 1BD2. This entry also has co-ordinates of the HLA-cognate peptide in complex with the TCR. The TCR chain B was the same in NY-ESO, but chain A had small differences in the C-domain and significant differences in the N-domain. Using the 1BD2 A/B model for molecular replacement, MR, gave an incorrect solution, as shown by extensive overlap with symmetry equivalent molecules. Using the B chain alone gave a better solution, which did not have significant clashes with neighbours. The

30

-60-

correlation coefficient was 49%, the crystallographic R-factor 50%, and the nearest approach (centre-of-gravity to c-o-g) was 0.49 nm (49Å). The rotation and translation operation needed to transform the starting chain B model to the MR equivalent, was applied to chain A. The hybrid MR solution thus generated, packed well in the cell,
5 with minimal clashes.

Electron density maps generally agreed with the model, and allowed its adjustment to match the sequence of the NY-ESO TCR. But the starting model had many gaps, specifically missing side-chains, that are characteristic of poorly ordered portions of
10 the model. Many of the hair-pin loops in between strands had very low density, and were difficult to model. The crystallographic R-factor of the model is 30%. The R-factor is a residual, i.e. it is the difference between the calculated and observed amplitudes.

15 As Figures 83a and 83b demonstrate, the input sequence from 1BD2 do not match up with the density very well. Changing the model for Cys at positions 164 in chain A, and 174 in chain B, followed by further refinement, showed clearly that this sequence assignment is much better fitted to the density. But the differences in terms of size of the side chain are minimal, so there was little perturbation in the model. The electron
20 density in that region is little changed.

The most important aspect of this work is that the new TCR is very similar in structure to the published model (1BD2). The comparison could include all of the TCR, the constant domains, or the small part near the mutation point.

25

The r.m.s deviation values are listed in the table below. The comparison of structures is shown in Figure 84.

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	Chain A Complete	Chain B Complete	Chain A Constant	Chain B Constant	Short Stretch
r.m.s Displacement	2.831	1.285	1.658	1.098	0.613
Mean Displacement	2.178	1.001	1.235	0.833	0.546
Max Displacement	9.885	6.209	6.830	4.490	1.330

(All units are in Å)

The short stretch refers to the single strand from Chain A (A157 to A169) and the single strand from Chain B (B170 to B183) that are now joined by the disulphide
5 bridge. The deviations were calculated for only the main chain atoms.

These results show that the introduction of the disulphide bond has minimal effect on the local structure of the TCR around the bond. Some larger effects are observed when comparing the TCR to the published structure (1BD2) of the A6 TCR, but the
10 increase in RMS displacement is largely due to differences in loop conformations (see Figure 84). These loops do not form part of the core structure of the TCR, which is formed by a series of β -sheets which form a characteristic Ig fold. The RMS deviation for the whole α -chain is particularly large because of the difference in the sequence of the variable domains between the A6 (1BD2) and the NY-ESO TCRs. However, the
15 A6 and NY-ESO TCRs have the same variable β -domain and the RMS deviations for the whole β -chain show that the structure of this variable domain is also maintained in the TCR with the new disulphide bond. These data therefore indicate that the core structure of the TCR is maintained in the crystal structure of the TCR with the new disulphide bond.

20

Example 10 – Production of soluble NY-ESO TCRs containing a novel disulphide inter-chain bond, and C-terminal β chain tagging sites.

In order to produce a soluble NY-ESO TCR incorporating a novel disulphide bond, A6
25 TCR plasmids containing the α chain BamHI and β chain BglII restriction sites were used as frameworks as described in Example 4.

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NY-ESO TCR β -chain constructs were obtained by PCR cloning as follows. PCR reactions were performed using the primers as shown below, and templates containing the NY-ESO TCR chains.

5

| NdeI |

Fwd5' -GGAGATATACATATGGGTGTCACTCAGAAC-3'

Rev5' -CCACCGGATCCGTCTGCTCTACCCCAGGC-3'

| BamHI |

10

The PCR products were restriction digested with the relevant restriction enzymes, and cloned into pGMT7 containing the biotin recognition sequence to obtain expression plasmids. The sequence of the plasmid inserts were confirmed by automated DNA sequencing. Figure 85a shows the DNA sequence of the β chain of the NY-ESO TCR incorporating the biotin recognition site, and Figure 85b shows the resulting amino acid sequence.

15

The α chain construct was produced as described in Example 5. The respective TCR chains were expressed, co-refolded and purified as described in Example 5.

20

In order to produce a soluble NY-ESO TCR containing a non-native disulphide inter-chain bond and a hexa-histidine tag on the C- terminus of the β chain, the same primers and NY-ESO template were used as above. The PCR products were restriction digested with the relevant restriction enzymes, and cloned into pGMT7 containing the hexa-histidine sequence to obtain expression plasmids. Figure 86a shows the DNA sequence of the β chain of the NY-ESO TCR incorporating the hexa-histidine tag, and Figure 86b shows the resulting amino acid sequence.

25

Figure 87 illustrates the elution of soluble NY-ESO TCR containing a novel disulphide bond and the biotin recognition sequence from a POROS 50HQ anion exchange column using a 0-500 mM NaCl gradient, as indicated by the dotted line. Figure 88 illustrates the elution of soluble NY-ESO TCR containing a novel

30

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disulphide bond and the hexa-histidine tag from a POROS 50HQ anion exchange columns using a 0-500 mM NaCl gradient, as indicated by the dotted line.

5 Figures 89 and 90 are protein elution profiles from gel filtration chromatography of pooled fractions from the NY-ESO-biotin and NY-ESO-hexa-histidine tagged anion exchange column runs illustrated by Figures 87 and 88 respectively. The protein elutes as a single major peak, corresponding to the TCR heterodimer.

10 A BIAcore analysis of sTCR binding to pMHC was carried out as described in Example 3. The NY-ESO-biotin TCR had a K_d of 7.5 μ M, The NY-ESO-hexa-histidine tagged TCR had a K_d of 9.6 μ M

Example 11 – Cell staining using fluorescent labelled tetramers of soluble NY-ESO TCR containing a novel disulphide inter-chain bond.

15

TCR Tetramer preparation

The NY-ESO soluble TCRs containing a novel disulphide bond and a biotin recognition sequence prepared as in Example 10 were utilised to form the soluble TCR tetramers using required for cell staining. 2.5 ml of purified soluble TCR solution (~
20 0.2 mg/ml) was buffer exchanged into biotinylation reaction buffer (50 mM Tris pH 8.0, 10 mM $MgCl_2$) using a PD-10 column (Pharmacia). The eluate (3.5 ml) was concentrated to 1 ml using a centricon concentrator (Amicon) with a 10 kDa molecular weight cut-off. This was made up to 10mM with ATP added from stock (0.1 g/ml adjusted to pH 7.0). A volume of a cocktail of protease inhibitors was then added
25 (protease inhibitor cocktail Set 1, Calbiochem Biochemicals), sufficient to give a final protease cocktail concentration of 1/100th of the stock solution as supplied, followed by 1 mM biotin (added from 0.2M stock) and 20 μ g/ml enzyme (from 0.5 mg/ml stock). The mixture was then incubated overnight at room temperature. Excess biotin was removed from the solution by size exclusion chromatography on a S75 HR
30 cloumn. The level of biotinylation present on the NY-ESO TCR was determined via a size exclusion HPLC-based method as follows. A 50ul aliquot of the biotinylated NY-ESO TCR (2mg/ml) was incubated with 50ul of streptavidin coated agarose beads

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(Sigma) for 1 hour. The beads were then spun down, and 50 μ l of the unbound sample was run on a TSK 2000 SW column (Tosohhaas) using a 0.5ml/min flowrate (200mM Phosphate Buffer pH 7.0) over 30 minutes. The presence of the biotinylated NY-ESO TCR was detected by a UV spectrometer at both 214nm and 280nm. The biotinylated NY-ESO was run against a non-bioninylated NY-ESO TCR control. The percentage of biotinylation was calculated by subtracting the peak-area of the biotinylated protein from that of the non-biotinylated protein.

Tetramerisation of the biotinylated soluble TCR was achieved using neutravidin-phycoerythrin conjugate (Cambridge Biosciences, UK). The concentration of biotinylated soluble TCR was measured using a Coomassie protein assay (Pierce), and a ratio of soluble TCR 0.8 mg/mg neutravidin-phycoerthrin conjugate was calculated to achieve saturation of the neutravidin-PE by biotinylated TCR at a ratio of 1:4. 19.5 μ l of a 6.15mg/ml biotinylated NY-ESO soluble TCR solution in phosphate buffered saline (PBS) was added slowly to 150 μ l of a 1mg/ml neutravidin-PE soluble over ice with gentle agitation. 100.5 μ l of PBS was then added to this solution to provide a final NY- ESO TCR tetramer concentration of 1 mg/ml.

Staining Protocol

Four aliquots of 0.3×10^6 HLA-A2 positive EBV transformed B cell line (PP LCL) in 0.5ml of PBS were incubated with varying concentrations (0 , 10^{-4} , 10^{-5} and 10^{-6} M) of HLA-A2 NYESO peptide (SLLMWITQC) for 2 h at 37°C . These PP LCL cells were then washed twice in Hanks buffered Saline solution (HBSS) (Gibco, UK).

Each of the four aliquots were divided equally and stained with biotinylated NY-ESO disulphide linked TCR freshly tetramerised with neutravidin-phycoerythrin. Cells were incubated with either 5 or 10 μ g of phycoerythrin labelled tetrameric dsTCR complexes on ice for 30 minutes and washed with HBSS. Cells were washed again, re-suspended in HBSS and analysed by FACSVantage. 25,000 events were collected and data analysed using WinMIDI software.

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Results

Figures 91a-h illustrate as histograms the FACS Vantage data generated for each of the samples prepared as described above. The following table lists the percentage of positively stained cells observed for each of the samples:

5

Sample	Positive stained Cells (%)
0 NY-ESO peptide, 5 μ g TCR	0.75
10 ⁻⁴ M NY-ESO peptide, 5 μ g TCR	84.39
10 ⁻⁵ M NY-ESO peptide, 5 μ g TCR	35.29
10 ⁻⁶ M NY-ESO peptide, 5 μ g TCR	7.98
0 NY-ESO peptide, 10 μ g TCR	0.94
10 ⁻⁴ M NY-ESO peptide, 10 μ g TCR	88.51
10 ⁻⁵ M NY-ESO peptide, 10 μ g TCR	8.25
10 ⁻⁶ M NY-ESO peptide, 10 μ g TCR	3.45

These data clearly indicate that the proportion of the cells labelled by the NY-ESO TCR tetramers increases in a manner correlated to the concentration of the peptide (SLLMWITQC) in which they had been incubated. Therefore, these NY-ESO TCR tetramers are moieties suitable for specific cell labelling based on the expression of the HLA-A2 NY-ESO complex.

In the present example, a fluorescent conjugated NY-ESO TCR tetramer has been used. However, similar levels of cell binding would be expected if this label were replaced by a suitable therapeutic moiety.

Example 12 – Production of soluble A6 TCR with a novel disulphide bond incorporating the C β 1 constant region.

All of the previous examples describe the production of soluble TCRs with a novel disulphide bond incorporating the C β 2 constant region. The present example

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demonstrates that soluble TCRs incorporating the C β 1 constant region can be produced successfully.

Design of primers for PCR stitching of A6 TCR β -chain V-domain to C β 1.

- 5 For PCR construct of A6 TCR β -chain V-domain, the following primers were designed:

5'-GGAGATATACATATGAACGCTGGTGTCAC-3'

5'-CCTTGTTTCAGGTCCTCTGTGACCGTGAG-3'

10

For PCR construct of C β 1, the following primers were designed:

5'-CTCACGGTCACAGAGGACCTGAACAAGG-3'

5'-CCCAAGCTTAGTCTGCTCTACCCCAGGCCTCGGC-3'

15

- Beta VTCR construct and C β 1 construct were separately amplified using standard PCR technology. They were connected to each other using a stitching PCR. Plasmid DNA was purified on a Qiagen mini-prep column according to the manufacturer's instructions and the sequence was verified by automated sequencing at the sequencing facility of Department of Biochemistry, Oxford University. The sequence for A6+C β 1 is shown in Figure 92.

20

Consequently, the A6+C β 1 chain was paired to A6 alpha TCR by inter-chain disulphide bond after introducing cysteine in C-domain of both chains.

25

The soluble TCR was expressed and refolded as described in Example 2.

Purification of refolded soluble TCR:

- sTCR was separated from degradation products and impurities by loading the dialysed
30 refold onto a POROS 50HQ anion exchange column and eluting bound protein with a gradient of 0-500mM NaCl over 50 column volumes using an Akta purifier

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(Pharmacia) as in Figure 93. Peak fractions were stored at 4°C and analysed by Coomassie-stained SDS-PAGE (Figure 94) before being pooled and concentrated. Finally, the sTCR was purified and characterised using a Superdex 200HR gel filtration column (Figure 95) pre-equilibrated in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a relative molecular weight of approximately 50 kDa was pooled and concentrated prior to characterisation by BIAcore surface plasmon resonance analysis.

A BIAcore analysis of the binding of the disulphide-linked A6 TCR to pMHC was carried out as described in Example 3. Figure 96 shows BIAcore analysis of the specific binding of disulphide-linked A6 soluble TCR to its cognate pMHC.

The soluble A6 TCR with a novel disulphide bond incorporating the Cβ1 constant region had a K_d of $2.42 \pm 0.55 \mu\text{M}$ for its cognate pMHC. This value is very similar to the K_d of $1.8 \mu\text{M}$ determined for the soluble A6 TCR with a novel disulphide bond incorporating the Cβ2 constant region as determined in Example 3.

Example 13 – Production of soluble A6 TCR with a novel disulphide bond incorporating the “free” cysteine in the β chain

20

The β chain constant regions of TCRs include a cysteine residue (residue 75 in exon 1 of TRBC1*01 and TRBC2*01) which is not involved in either inter-chain or intra-chain disulphide bond formation. All of the previous examples describe the production of soluble TCRs with a novel disulphide bond in which this “free” cysteine has been mutated to alanine in order to avoid the possible formation of any “inappropriate” disulphide bonds which could result in a reduced yield of functional TCR. The present example demonstrates that soluble TCRs incorporating this “free” cysteine can be produced.

25

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Design of primers and mutagenesis of TCR β chain

For mutating TCR β -chain alanine (residue 75 in exon 1 of TRBC1*01 and TRBC2*01) to cysteine, the following primers were designed (mutation shown in lower case):

5

5'-T GAC TCC AGA TAC tgT CTG AGC AGC CG

5'-CG GCT GCT CAG Aca GTA TCT GGA GTC A

10 PCR mutagenesis, expression and refolding of the soluble TCR was carried out as described in Example 2.

Purification of refolded soluble TCR:

sTCR was separated from degradation products and impurities by loading the dialysed
 15 refold onto a POROS 50HQ anion exchange column and eluting bound protein with a
 gradient of 0-500mM NaCl over 50 column volumes using an Akta purifier
 (Pharmacia) as in Figure 98. Peak fractions were stored at 4°C and analysed by
 Coomassie-stained SDS-PAGE (Figure 99) before being pooled and concentrated.
 Finally, the sTCR was purified and characterised using a Superdex 200HR gel
 20 filtration column (Figure 100) pre-equilibrated in HBS-EP buffer (10 mM HEPES pH
 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a relative
 molecular weight of approximately 50 kDa was pooled and concentrated prior to
 characterisation by BIAcore surface plasmon resonance analysis.

25 A BIAcore analysis of the binding of the disulphide-linked A6 TCR to pMHC was
 carried out as described in Example 3. Figure 101 shows BIAcore analysis of the
 specific binding of disulphide-linked A6 soluble TCR to its cognate pMHC.

The soluble A6 TCR with a novel disulphide bond incorporating the "free" cysteine in
 the β chain had a K_d of $21.39 \pm 3.55 \mu\text{M}$ for its cognate pMHC.

30

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Example 14 – Production of soluble A6 TCR with a novel disulphide bond wherein “free” cysteine in the β chain is mutated to serine .

The present example demonstrates that soluble TCRs with a novel disulphide bond in which the “free” cysteine in the β chain (residue 75 in exon 1 of TRBC1*01 and TRBC2*01) is mutated to serine can be successfully produced.

Design of primers and mutagenesis of TCR β chain

For mutating TCR β -chain alanine that had previously been substituted for the native cysteine (residue 75 in exon 1 of TRBC1*01 and TRBC2*01) to serine, the following primers were designed (mutation shown in lower case):

5'-T GAC TCC AGA TAC tCT CTG AGC AGC CG

5'-CG GCT GCT CAG AGa GTA TCT GGA GTC A

15

PCR mutagenesis (resulting in a mutated beta chain as shown in Figure 102), expression and refolding of soluble TCR was carried out as described in Example 2.

Purification of refolded soluble TCR:

sTCR was separated from degradation products and impurities by loading the dialysed refold onto a POROS 50HQ anion exchange column and eluting bound protein with a gradient of 0-500mM NaCl over 50 column volumes using an Akta purifier (Pharmacia) as shown in Figure 103. Peak fractions were stored at 4°C and analysed by Coomassie-stained SDS-PAGE (Figure 104) before being pooled and concentrated. Finally, the sTCR was purified and characterised using a Superdex 200HR gel filtration column (Figure 105) pre-equilibrated in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a relative molecular weight of approximately 50 kDa was pooled and concentrated prior to characterisation by BIAcore surface plasmon resonance analysis.

30

-70-

A BIAcore analysis of the binding of the disulphide-linked A6 TCR to pMHC was carried out as described in Example 3. Figure 106 shows BIAcore analysis of the specific binding of disulphide-linked A6 soluble TCR to its cognate pMHC.

- 5 The soluble A6 TCR with a novel disulphide bond in which the “free” cysteine in the β chain was mutated to serine had a K_d of $2.98 \pm 0.27 \mu\text{M}$ for its cognate pMHC. This value is very similar to the K_d of $1.8 \mu\text{M}$ determined for the soluble A6 TCR with a novel disulphide bond in which the “free” cysteine in the β chain was mutated to alanine as determined in Example 3.

10

Example 15 – Cloning of NY-ESO TCR α and β chains containing a novel disulphide bond into yeast expression vectors

- NY-ESO TCR α and β chains were fused to the C-terminus of the pre-pro mating factor alpha sequence from *Saccharomyces cerevisiae* and cloned into yeast expression vectors pYX122 and pYX112 respectively (see Figures 107 and 108).

- The following primers were designed to PCR amplify pre-pro mating factor alpha sequence from *S. cerevisiae* strain SEY6210 (Robinson *et al.* (1991), *Mol Cell Biol.* 11(12):5813-24) for fusing to the TCR α chain.

5' -TCT GAA TTC ATG AGA TTT CCT TCA ATT TTT AC-3'

5' -TCA CCT CCT GGG CTT CAG CCT CTC TTT TAT C -3'

- 25 The following primers were designed to PCR amplify pre-pro mating factor alpha sequence from *S. cerevisiae* strain SEY6210 for fusing to the TCR β chain.

5' -TCT GAA TTC ATG AGA TTT CCT TCA ATT TTT AC-3'

5' -GTG TCT CGA GTT AGT CTG CTC TAC CCC AGG C-3'

30

Yeast DNA was prepared by re-suspending a colony of *S. cerevisiae* strain SEY6210 in $30\mu\text{l}$ of 0.25% SDS in water and heating for 3 minutes at 90°C . The pre-pro mating

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factor alpha sequences for fusing to the TCR α and β chains were generated by PCR amplifying 0.25 μ l of yeast DNA with the respective primer pairs mentioned above using the following PCR conditions. 12.5pmoles of each primer was mixed with 200 μ M dNTP, 5 μ l of 10x Pfu buffer and 1.25units of Pfu polymerase (Stratagene) in a
 5 final volume of 50 μ l. After an initial denaturation step of 30 seconds at 92°C, the reaction mixture was subjected to 30 rounds of denaturation (92°C, 30 sec.), annealing (46.9°C, 60 sec.), and elongation (72°C, 2 min.) in a Hybaid PCR express PCR machine.

10 The following primers were designed to PCR amplify the TCR α chain to be fused to the pre-pro mating factor alpha sequence mentioned above.

5' -GGC TGA AGC CCA GGA GGT GAC ACA GAT TCC-3'

5' -CTC CTC TCG AGT TAG GAA CTT TCT GGG CTG GG-3'

15

The following primers were designed to PCR amplify the TCR β chain to be fused to the pre-pro mating factor alpha sequence mentioned above.

5' -GGC TGA AGC CGG CGT CAC TCA GAC CCC AAA AT-3'

20 5' -GTG TCT CGA GTT AGT CTG CTC TAC CCC AGG C-3'

The PCR conditions for amplifying the TCR α and β chains were the same as mentioned above except for the following changes: the DNA template used for amplifying the TCR α and β chains were the NY-ESO TCR α and β chains
 25 respectively (as prepared in Example 5); and the annealing temperature used was 60.1°C.

The PCR products were then used in a PCR stitching reaction utilising the complementary overlapping sequences introduced into the initial PCR products to
 30 create a full length chimeric gene. The resulting PCR products were digested with the restriction enzymes EcoR I and Xho I and cloned into either pYX122 or pYX112 digested with the same enzymes. The resulting plasmids were purified on a Qiagen™

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mini-prep column according to the manufacturer's instructions, and the sequences verified by automated sequencing at the sequencing facility of Genetics Ltd, Queensway, New Milton, Hampshire, United Kingdom. Figures 109 and 110 show the DNA and protein sequences of the cloned chimeric products.

5

Example 16 – Expression of soluble NY-ESO TCR containing a novel disulphide bond in yeast

The yeast expression plasmids containing the TCR α and β chains respectively
10 produced as described in Example 15 were co-transformed into *S. cerevisiae* strain SEY6210 using the protocol by Agatep *et al.* (1998) (Technical Tips Online (<http://tto.trends.com>) 1:51:P01525). A single colony growing on synthetic dropout (SD) agar containing Histidine and Uracil (Qbiogene, Illkirch, France) was cultured overnight at 30°C in 10ml SD media containing Histidine and Uracil. The overnight
15 culture was sub-cultured 1:10 in 10ml of the fresh SD media containing Histidine and Uracil and grown for 4 hours at 30°C. The culture was centrifuged for 5 minutes at 3800rpm in a Heraeus Megafuge 2.0R (Kendro Laboratory Products Ltd, Bishop's Stortford, Hertfordshire, UK) and the supernatant harvested. 5 μ l StratClean Resin (Stratagene) was mixed with the supernatant and kept rotating in a blood wheel at 4°C
20 overnight. The StrataClean resin was spun down at 3800rpm in a Heraeus Megafuge 2.0R and the media discarded. 25 μ l of reducing sample buffer (950 μ l of Laemmli sample buffer (Biorad) containing 50 μ l of 2M DTT) was added to the resin and the samples heated at 95°C for 5 minutes and then cooled on ice before 20 μ l of the mix was loaded on a SDS-PAGE gel at 0.8mA constant /cm² of gel surface for 1 hour.
25 The proteins in the gel were transferred to Immuno-Blot PVDF membranes (Bio-Rad) and probed with TCR anti α chain antibody as described in Example 17 below except for the following changes. The primary antibody (TCR anti α chain) and secondary antibodies were used at 1 in 200 and 1 in 1000 dilutions respectively. Figure 111 shows a picture of the developed membrane. The result shows that there is a low level
30 of TCR secretion by the yeast culture into the media.

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*Example 17 - Disulphide A6 Tax TCR α and β chain expression in Baculovirus**Strategy for cloning*

The α and β chains of the disulphide A6 Tax TCR were cloned from pGMT7 into a pBlueScript KS2- based vector called the pEX172. This vector was designed for cloning different MHC class II β -chains, for insect cell expression, using the leader sequence from DRB1*0101, an AgeI site for insertion of different peptide-coding sequences, a linker region, and then MluI and SalI sites to clone the DR β chains in front of the Jun Leucine zipper sequence. The sequence where pEX 172 differs from pBlueScript II KS-, located between the KpnI and EcoRI sites of pBlueScript II KS-, is shown in Figure 112. For the purposes of cloning TCR chains in insect cells, this pEX172 was cut with AgeI and SalI to remove the linker region and MluI site, and the TCR chains go in where the peptide sequence would start. The TCR sequences were cloned from pGMT7 with a BspEI site at the 5' end (this had AgeI compatible sticky ends) and a SalI site at the 3' end. In order to provide the cleavage site for the removal of the DR β leader sequence, the first three residues of the DR β chain (GDT) were preserved. In order to prevent the Jun Leucine zipper sequence being transcribed, it was necessary to insert a stop codon before the SalI site. For a schematic of this construct, see Figure 113. Once the TCR chains are in this plasmid, the BamHI fragment were cut out and subcloned into the pAcAB3 vector, which has homology recombination sites for Baculovirus. The pAcAB3 vector has two divergent promoters, one with a BamHI site and one with a BglII cloning site. There is a BglII site in the A6 TCR β -chain, so the A6 TCR α -chain was inserted into the BglII site, and the β -chain was then subcloned into the BamHI site.

25

In accordance with the above cloning strategy, the following primers were designed (homology to the vectors is in uppercase):

A6 α : F: 5'-gtagtccggagacaccggaCAGAAGGAAGTGGAGCAGAAC
 30 R: 5'-gtagggtcgacTAGGAACTTTCTGGGCTGGG
 A6 β : F: 5'-gtagtccggagacaccggaAACGCTGGTGTCACCTCAGA

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R: 5' -gtaggtcgactAGTCTGCTCTACCCCAGG

PCR, cloning and sub-cloning:

Expression plasmids containing the genes for the disulphide A6 Tax TCR α or β chain were used as templates in the following PCR reactions. 100ng of α plasmid was mixed with 1 μ l 10mM dNTP, 5 μ l 10xPfu-buffer (Stratagene), 1.25 units Pfu polymerase (Stratagene), 50pmol of the A6 α primers above, and the final volume was adjusted to 50 μ l with H₂O. A similar reaction mixture was set up for the β chain, using the β plasmid and the pair of β primers. The reaction mixtures were subjected to 35 rounds of denaturation (95°C, 60 sec.), annealing (50°C, 60 sec.), and elongation (72°C, 8 min.) in a Hybaid PCR express PCR machine. The product was then digested for 2 hours at 37°C with 10 units of BspEI restriction enzyme then for a further 2 hours with 10 units of Sall (New England Biolabs). These digested reactions were ligated into pEX172 that had been digested with AgeI and Sall, and these were transformed into competent XL1-Blue bacteria and grown for 18 hours at 37°C. A single colony was picked from each of the α and β preps and grown over night in 5 ml TYP + ampicillin (16 g/l Bacto-Tryptone, 16 g/l Yeast Extract, 5 g/l NaCl, 2.5 g/l K₂HPO₄, 100 mg/l Ampicillin). Plasmid DNA was purified on a QIAgen mini-prep column according to the manufacturer's instructions and the sequence was verified by automated sequencing at the sequencing facility of Genetix. The amino acid sequences of the BamHI inserts are shown in Figures 114 and 115 for the α chain and β chain, respectively.

These α and β disulphide A6 Tax TCR chain constructs in pEX172 were digested out for 2 hours at 37°C with BamHI restriction enzyme (New England Biolabs). The α chain BamHI insert was ligated into pAcAB3 vector (PharMingen-BD Biosciences: 21216P) that had been digested with BglII enzyme. This was transformed into competent XL1-Blue bacteria and grown for 18 hours at 37°C. A single colony was picked from this plate and grown overnight in 5 ml TYP + ampicillin and the plasmid DNA was purified as before. This plasmid was then digested with BamHI and the β chain BamHI insert was ligated in, transformed into competent XL1-Blue bacteria,

-75-

grown overnight, picked to TYP-ampicillin, and grown before miniprepping as before using a QIAgen mini-prep column. The correct orientation of both the α and β chains were confirmed by sequencing using the following sequencing primers:

- 5 pAcAB3 α forwards: 5'-gaaattatgcatttgaggatg
pAcAB3 β forwards: 5'-attaggcctctagagatccg

Transfection, infection, expression and analysis of A6 TCR in insect cells

- The expression plasmid containing the α -chain and β -chain was transfected into sf9
10 cells (Pharmingen-BD Biosciences: 21300C) grown in serum free medium
(Pharmingen-BD Biosciences: 551411), using the Baculogold transfection kit
(Pharmingen-BD Biosciences: 21100K) as per the manufacturers instructions. After 5
days at 27°C, 200 μ l of the medium these transfected cells had been growing in was
added to 100ml of High Five cells at 1x10⁶ cells/ml in serum free medium. After a
15 further 6 days at 27°C, 1ml of this medium was removed and centrifuged at
13,000RPM in a Hereus microfuge for 5 minutes to pellet cell debris.

- 10 μ l of this insect A6 disulphide linked TCR supernatant was run alongside positive
controls of bacterial A6 disulphide linked TCR 5 μ g and 10 μ g on a pre-cast 4-20%
20 Tris/glycine gel (Invitrogen: EC60252). Reduced samples were prepared by adding
10 μ l of Reducing sample buffer (950 μ l of Laemmli sample buffer (Bio-Rad: 161-
0737) 50 μ l of 2M DTT) and heating at 95°C for 5 minutes, cooling at room
temperature for 10 minutes then loading 20 μ l. Non-reduced samples were prepared by
adding 10 μ l of Laemmli sample buffer, and loading 20 μ l.

25

- The gel was run at 150 volts for 1 hour in a Novex - Xcell gel tank after which the gel
was stained in 50ml of Coomassie gel stain for 1 hour with gentle agitation (1.1g
Coomassie powder in 500ml of methanol stir for 1 hour add 100ml acetic acid make
up to 1 litre with H₂O and stir for 1 hour then filter through 0.45 μ M filter). The gel
30 was de-stained three times for 30 mins with gentle agitation in 50ml of de-stain (as
Coomassie gel stain but omitting the Coomassie powder).

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Western Blots were performed by running SDS-PAGE gels as before but the proteins were transferred to Immuno-Blot PVDF membranes (Bio-Rad: 162-0174) rather than staining the gels with Coomassie. Six filter papers were cut to the size of the gel and
5 soaked in transfer buffer (2.39g Glycine, 5.81g of Tris Base, 0.77g DTT dissolved in 500mls of H₂O, 200mls of methanol added then made up to 1000mls with H₂O). The PVDF membrane was prepared by soaking in methanol for 1 minute and then in transfer buffer for 2 minutes. Three filter papers were placed on the anode surface of the Immno-blot apparatus (Pharmacia – Novablot) then the membrane was placed on
10 top followed by the gel and then finally three more filter papers on the cathode side. The Immuno-blot was run for 1 hour at 0.8mA constant /cm² of gel surface.

After blotting, the membrane was blocked in 7.5mls of blocking buffer (4 Tris-buffered saline tablets (Sigma: T5030), 3g non-fat dried milk (Sigma: M7409), 30µl of
15 Tween 20 made up to 30mls with H₂O) for 60 mins with gentle agitation. The membrane was washed three times for 5 mins with TBS wash buffer (20 TBS tablets, 150µl Tween 20 made up to 300ml with H₂O). The membrane was then incubated in primary antibody 1 in 50 dilution of anti TCR α chain clone 3A8 (Serotec: MCA987) or anti TCR β chain clone 8A3 (Serotec: MCA988) in 7.5ml blocking buffer for 1
20 hour with gentle agitation. The membrane was washed as before in TBS wash buffer. Next, a secondary antibody incubation of HRP labelled goat anti-mouse antibody (Santa Cruz Biotech: Sc-2005) 1 in 1000 dilution in 7.5ml of blocking buffer was carried out for 30 min with gentle agitation. The membrane was washed as before and then washed in 30ml of H₂O with 2 TBS tablets.

25

The antibody binding was detected by Opti-4CN colourmetric detection (Biorad: 170-8235) (1.4ml Opt-4CN diluent, 12.6ml H₂O, 0.28ml Opti-4CN substrate). The membranes were coloured for 30 minutes and then washed in H₂O for 15 minutes. The membranes were dried at room temperature, and scanned images were aligned
30 with an image of the coomassie stained gel (Figure 116).

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Results

- It can be seen from Figure 116 that both disulphide TCRs are formed as a heterodimer that is stable in the SDS gel. They both break into the α and β chains upon reduction. The insect disulphide TCR heterodimer has a slightly higher molecular weight than the
- 5 bacterially produced version, presumably because of the glycosylation from the insect cells. It can be seen that in this instance the insect cells are producing α chain in excess, and free α chain can be seen in the non-reduced lane of the anti- α western blot.
- 10 These data clearly demonstrate that the baculovirus expression system described above provides a viable alternative to prokaryotic expression of soluble TCRs containing novel disulphide bonds.

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SEQUENCE LISTING

<110> Avidex Limited

<120> Soluble T Cell Receptor

<130> 80514-41

<140> PCT/GB02/03986

<141> 2002-08-30

<150> GB 0121187.9

<151> 2001-08-31

<150> GB0219146.8

<151> 2002-08-16

<150> US 60/404182

<151> 2002-08-16

<160> 183

<170> PatentIn version 3.1

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<211> 20

<212> PRT

<213> Homo sapiens

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<211> 20

<212> PRT

<213> Homo sapiens

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Met	Arg	Ser	Met
			20

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Gln Pro Ala Leu
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Ile Asn Val Pro Lys Thr Met Glu Ser Gly Thr Phe Ile Thr Asp Lys
35 40 45

Thr Val Leu Asp Met Lys Ala Met Asp Ser Lys Ser Asn Gly Ala Ile
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Ala Trp Ser Asn Gln Thr Ser Phe Thr Cys Gln Asp Ile Phe Lys Glu
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Thr Asn Ala Thr Tyr Pro Ser Ser Asp Val Pro
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35 40 45

Gly Arg Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Ala Tyr Lys
50 55 60

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Glu Ser Asn Tyr Ser Tyr Cys Leu Ser Ser Arg Leu Arg Val Ser Ala
65 70 75 80

Thr Phe Trp His Asn Pro Arg Asn His Phe Arg Cys Gln Val Gln Phe
85 90 95

His Gly Leu Ser Glu Glu Asp Lys Trp Pro Glu Gly Ser Pro Lys Pro
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Val Thr Pro Pro Lys Val Ser Leu Phe Glu Pro Ser Lys Ala Glu Ile
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aacgtgttcc	cacccgaggt	cgctgtgttt	gagccatcag	aagcagagat	ctcccacacc	420
caaaaggcca	cactgggtgtg	cctggccaca	ggcttctacc	ccgaccacgt	ggagctgagc	480

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accttctggc	aggacccccg	caaccacttc	cgctgtcaag	tccagttcta	cgggctctcg	660
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<400> 109

Asn	Ala	Gly	Val	Thr	Gln	Thr	Pro	Lys	Phe	Gln	Val	Leu	Lys	Thr	Gly	
1				5					10					15		
Gln	Ser	Met	Thr	Leu	Gln	Cys	Ala	Gln	Asp	Met	Asn	His	Glu	Tyr	Met	
			20					25					30			
Ser	Trp	Tyr	Arg	Gln	Asp	Pro	Gly	Met	Gly	Leu	Arg	Leu	Ile	His	Tyr	
		35					40					45				
Ser	Val	Gly	Ala	Gly	Ile	Thr	Asp	Gln	Gly	Glu	Val	Pro	Asn	Gly	Tyr	
	50					55					60					
Asn	Val	Ser	Arg	Ser	Thr	Thr	Glu	Asp	Phe	Pro	Leu	Arg	Leu	Leu	Ser	
65					70					75					80	
Ala	Ala	Pro	Ser	Gln	Thr	Ser	Val	Tyr	Phe	Cys	Ala	Ser	Arg	Pro	Gly	
				85					90					95		
Leu	Ala	Gly	Gly	Arg	Pro	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	
			100					105					110			
Thr	Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala	Val	
		115					120					125				
Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	Leu	
	130					135					140					
Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser	Trp	
145					150					155				160		
Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro	Gln	
				165					170					175		
Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Ala	Leu	Ser	
			180					185					190			
Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asp	Pro	Arg	Asn	His	
		195					200					205				
Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	Trp	
	210					215					220					
Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	Ala	
225					230					235					240	

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Trp Gly Arg Ala Asp
245

<210> 110
<211> 621
<212> DNA
<213> Homo sapiens

<400> 110
atgcagaagg aagtggagca gaactctgga cccctcagtg ttccagaggg agccattgcc 60
tctctcaact gcacttacag tgaccgaggt tcccagtcct tcttctggta cagacaatat 120
tctgggaaaa gccctgagtt gataatgtcc atatactcca atggtgacaa agaagatgga 180
aggtttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg 300
cagtttggag cagggaccca ggttgtggtc accccagata tccagaacct ggatcctgcc 360
gtgtaccagc tgagagactc taaatccagt gacaagtctg tctgcctatt caccgatttt 420
gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaatgt 480
gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc 600
cccagcccag aaagttccta a 621

<210> 111
<211> 615
<212> DNA
<213> Homo sapiens

<400> 111
atgcaactac tagaaciaag tcctcagttt ctaagcatcc aagagggaga aaatctcact 60
gtgtactgca actcctcaag tgttttttcc agcttacaat ggtacagaca ggagcctggg 120
gaaggctcctg tcctcctggt gacagtagtt acgggtggag aagtgaagaa gctgaagaga 180
ctaacctttc agtttggtga tgcaagaaag gacagttctc tccacatcac tgcggcccag 240
cctggtgata caggcctcta cctctgtgca ggagcgggaa gccaaaggaaa tctcatcttt 300
ggaaaaggca ctaaactctc tgttaaacca aatatccaga acccggatcc tgccgtgtac 360
cagctgagag actctaaatc cagtgcacaag tctgtctgcc tattcaccga ttttgattct 420
caaacaaatg tgtcacaag taaggattct gatgtgtata tcacagacaa atgtgtgcta 480
gacatgaggt ctatggactt caagagcaac agtgctgtgg cctggagcaa caaatctgac 540
tttgcattgt caaacgcctt caacaacagc attattccag aagacacctt ctccccagc 600
ccagaaagtt cctaa 615

<210> 112
<211> 735
<212> DNA
<213> Homo sapiens

<400> 112
atgggtggatg gtggaatcac tcagtcccca aagtacctgt tcagaaagga aggacagaat 60
gtgaccctga gttgtgaaca gaatttgaac cacgatgcca tgtactggta ccgacaggac 120
ccagggaag ggctgagatt gatctactac tcacagatag taaatgactt tcagaaagga 180
gatatagctg aagggtacag cgtctctcgg gagaagaagg aatcctttcc tctcactgtg 240
acatcgcccc aaaagaacct gacagctttc tatctctgtg ccagtagttc gaggagctcc 300
tacgagcagt acttcggggc gggcaccagg ctacagggtc cagaggacct gaaaaacgtg 360
ttcccacccg aggtegtgtg gtttgagcca tcagaagcag agatctccca caccctaaag 420
gccacactgg tgtgcctggc cacaggcttc taccctgacc acgtggagct gagctggtgg 480
gtgaatggga aggaggtgca cagtggggtc tgcacagacc cgcagcccct caaggagcag 540
cccgccctca atgactccag atacagcctg agcagccgcc tgagggtctc ggccaccttc 600
tggcagaacc ccgcaacca cttccgtgt caagtccagt tctacgggct ctcggagaat 660
gacgagtgga ccaggatag ggccaaacct gtcacccaga ttgtcagcgc cgaggcctgg 720
ggtagagcag actaa 735

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<210> 113
 <211> 204
 <212> PRT
 <213> Homo sapiens

<400> 113
 Met Gln Leu Leu Glu Gln Ser Pro Gln Phe Leu Ser Ile Gln Glu Gly
 1 5 10 15
 Glu Asn Leu Thr Val Tyr Cys Asn Ser Ser Ser Val Phe Ser Ser Leu
 20 25 30
 Gln Trp Tyr Arg Gln Glu Pro Gly Glu Gly Pro Val Leu Leu Val Thr
 35 40 45
 Val Val Thr Gly Gly Glu Val Lys Lys Leu Lys Arg Leu Thr Phe Gln
 50 55 60
 Phe Gly Asp Ala Arg Lys Asp Ser Ser Leu His Ile Thr Ala Ala Gln
 65 70 75 80
 Pro Gly Asp Thr Gly Leu Tyr Leu Cys Ala Gly Ala Gly Ser Gln Gly
 85 90 95
 Asn Leu Ile Phe Gly Lys Gly Thr Lys Leu Ser Val Lys Pro Asn Ile
 100 105 110
 Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser Ser
 115 120 125
 Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn Val
 130 135 140
 Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val Leu
 145 150 155 160
 Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp Ser
 165 170 175
 Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile Ile
 180 185 190
 Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200

<210> 114
 <211> 244
 <212> PRT
 <213> Homo sapiens

<400> 114
 Met Val Asp Gly Gly Ile Thr Gln Ser Pro Lys Tyr Leu Phe Arg Lys
 1 5 10 15
 Glu Gly Gln Asn Val Thr Leu Ser Cys Glu Gln Asn Leu Asn His Asp
 20 25 30

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Ala Met Tyr Trp Tyr Arg Gln Asp Pro Gly Gln Gly Leu Arg Leu Ile
35 40 45

Tyr Tyr Ser Gln Ile Val Asn Asp Phe Gln Lys Gly Asp Ile Ala Glu
50 55 60

Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Ser Phe Pro Leu Thr Val
65 70 75 80

Thr Ser Ala Gln Lys Asn Pro Thr Ala Phe Tyr Leu Cys Ala Ser Ser
85 90 95

Ser Arg Ser Ser Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr
100 105 110

Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe
115 120 125

Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val
130 135 140

Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp
145 150 155 160

Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro
165 170 175

Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ser Leu Ser Ser
180 185 190

Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe
195 200 205

Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr
210 215 220

Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp
225 230 235 240

Gly Arg Ala Asp

<210> 115

<211> 627

<212> DNA

<213> Homo sapiens

<400> 115

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atgcaggagg ygacacagat tcctgcagct ctgagtgtcc cagaaggaga aaacttggtt      60
ctcaactgca gtttcactga tagcgctatt tacaacctcc agtggttttag gcaggaccct      120
gggaaaggtc tcacatctct gttgcttatt cagtcaagtc agagagagca aacaagtgga      180
agacttaatg cctcgctgga taaatcatca ggacgtagta ctttatacat tgcagcttct      240
cagcctgggtg actcagccac ctacctctgt gctgtgaggc ccacatcagg aggaagctac      300
atacctacat ttggaagagg aaccagcctt attgttcatt cgtatatcca gaaccctgac      360
cctgccgtgt accagctgag agactctaaa tccagtgaca agtctgtctg cctattcacc      420
gatttttgatt ctcaaacaaa tgtgtcacia agtaaggatt ctgatgtgta tatcacagac      480
aaatgtgtgc tagacatgag gtctatggac ttcaagagca acagtgtgtg ggcctggagc      540
aacaaatctg actttgcatg tgcaaacgcc ttcaacaaca gcattattcc agaagacacc      600

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ttcttcccca gccagaaag ttcctaa

627

<210> 116

<211> 729

<212> DNA

<213> Homo sapiens

<400> 116

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atgggtgtca ctcagacccc aaaattccag gtcctgaaga caggacagag catgacactg    60
cagtgtgccc aggatatgaa ccatgaatac atgtcctggt atcgacaaga cccaggcatg    120
gggctgaggc tgattcatta ctcagttggt gctggatatca ctgaccaagg agaagtcccc    180
aatggctaca atgtctccag atcaaccaca gaggatttcc cgctcaggct gctgtcggct    240
gctccctccc agacatctgt gtacttctgt gccagcagtt acgtcgggaa caccggggag    300
ctgttttttg gagaaggctc taggctgacc gtactggagg acctgaaaaa cgtgttccca    360
cccgaggctg ctgtgtttga gccatcagaa gcagagatct cccacacca aaaggccaca    420
ctgggtgtgcc tggccacagg cttctacccc gaccacgtgg agctgagctg gtgggtgaat    480
gggaaggagg tgcacagtgg ggtctgcaca gaccgcagc ccctcaagga gcagcccgcc    540
ctcaatgact ccagatacgc tctgagcagc cgcctgaggg tctcggccac cttctggcag    600
gacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag    660
tggaaccagg atagggccaa acccgtcacc cagatcgtea gcgccgaggc ctggggtaga    720
gcagactaa                                     729

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<210> 117

<211> 208

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<222> (4)..(4)

<223> X is any amino acid

<400> 117

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Met Gln Glu Xaa Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly
1              5              10              15

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
                20              25              30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35              40              45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50              55              60

Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser
65              70              75              80

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85              90              95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100             105             110

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp
115             120             125

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Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser
 130 135 140

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp
 145 150 155 160

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala
 165 170 175

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn
 180 185 190

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 118

<211> 244

<212> PRT

<213> Homo sapiens

<400> 118

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
 1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
 20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Glu Thr Gly Leu Arg Leu Ile His
 35 40 45

Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60

Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80

Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr
 85 90 95

Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr
 100 105 110

Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe
 115 120 125

Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val
 130 135 140

Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp
 145 150 155 160

Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro
 165 170 175

Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser
 180 185 190

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Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe
 195 200 205

Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr
 210 215 220

Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp
 225 230 235 240

Gly Arg Ala Asp

<210> 119
 <211> 630
 <212> DNA
 <213> Homo sapiens

<400> 119
 atgcaggagg ygacacagat tcctgcagct ctgagtgtcc cagaaggaga aaacttggtt 60
 ctcaactgca gtttcactga tagcgctatt tacaacctcc agtggtttag gcaggaccct 120
 gggaaaggtc tcacatctct gttgcttatt cagtcaagtc agagagagca aacaagtgga 180
 agacttaatg cctcgctgga taaatcatca ggacgtagta ctttatacat tgcagcttct 240
 cagcctgggtg actcagccac ctacctctgt gctgtgaggg ccacatcagg aggaagctac 300
 atacctacat ttggaagagg aaccagcctt attgttcctc cgtatatcca gaaccctgac 360
 cctgccgtgt accagctgag agactctaaa tccagtgaca agtctgtctg cctattcacc 420
 gattttgatt ctcaaacaaa tgtgtcacia agtaaggatt ctgatgtgta tatcacagac 480
 aaatgtgtgc tagacatgag gtctatggac ttcaagagca acagtgtctg ggcctggagc 540
 aacaaatctg actttgcatg tgcaaacgcc ttcaacaaca gcattattcc agaagacacc 600
 ttcttcccca gcccgaaaag ttctgtttaa 630

<210> 120
 <211> 732
 <212> DNA
 <213> Homo sapiens

<400> 120
 atgggtgtca ctcagacccc aaaattccag gtcctgaaga caggacagag catgacactg 60
 cagtgtgccc aggatatgaa ccatgaatac atgtcctggt atcgacaaga cccaggcatg 120
 gggctgaggg tgattcatta ctcagttggt gctgggtatca ctgaccaagg agaagtcccc 180
 aatggctaca atgtctccag atcaaccaca gaggatttcc cgctcaggct gctgtcggct 240
 gctccctccc agacatctgt gtacttctgt gccagcagtt acgtcgggaa caccggggag 300
 ctgttttttg gagaaggctc taggctgacc gtactggagg acctgaaaaa cgtgttccca 360
 cccgaggctg ctgtgtttga gccatcagaa gcagagatct cccacacca aaaggccaca 420
 ctgggtgtgcc tggccacagg cttctacccc gaccacgtgg agctgagctg gtgggtgaat 480
 gggaaggagg tgcacagtgg ggtctgcaca gacccgcagc ccctcaagga gcagcccgcc 540
 ctcaatgact ccagatacgc tctgagcagc cgctgaggg tctcggccac cttctggcag 600
 gacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag 660
 tggacccagg atagggccaa acccgtcacc cagatcgtca gcgccgaggg ctggggtaga 720
 gcagactgtt aa 732

<210> 121
 <211> 209
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (4)..(4)

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<223> X is any amino acid

<400> 121

Met	Gln	Glu	Xaa	Thr	Gln	Ile	Pro	Ala	Ala	Leu	Ser	Val	Pro	Glu	Gly
1				5					10					15	
Glu	Asn	Leu	Val	Leu	Asn	Cys	Ser	Phe	Thr	Asp	Ser	Ala	Ile	Tyr	Asn
			20					25					30		
Leu	Gln	Trp	Phe	Arg	Gln	Asp	Pro	Gly	Lys	Gly	Leu	Thr	Ser	Leu	Leu
		35					40					45			
Leu	Ile	Gln	Ser	Ser	Gln	Arg	Glu	Gln	Thr	Ser	Gly	Arg	Leu	Asn	Ala
	50					55					60				
Ser	Leu	Asp	Lys	Ser	Ser	Gly	Arg	Ser	Thr	Leu	Tyr	Ile	Ala	Ala	Ser
65					70					75					80
Gln	Pro	Gly	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Arg	Pro	Thr	Ser
				85					90					95	
Gly	Gly	Ser	Tyr	Ile	Pro	Thr	Phe	Gly	Arg	Gly	Thr	Ser	Leu	Ile	Val
			100					105					110		
His	Pro	Tyr	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp
		115					120					125			
Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser
	130					135					140				
Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp
145					150					155					160
Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala
				165					170					175	
Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn
			180					185					190		
Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser
		195					200					205			

Cys

<210> 122

<211> 243

<212> PRT

<213> Homo sapiens

<400> 122

Met	Gly	Val	Thr	Gln	Thr	Pro	Lys	Phe	Gln	Val	Leu	Lys	Thr	Gly	Gln
1				5					10					15	
Ser	Met	Thr	Leu	Gln	Cys	Ala	Gln	Asp	Met	Asn	His	Glu	Tyr	Met	Ser
			20					25					30		

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Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
 35 40 45
 Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
 50 55 60
 Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
 65 70 75 80
 Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
 85 90 95
 Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
 100 105 110
 Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
 115 120 125
 Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
 130 135 140
 Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
 145 150 155 160
 Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
 165 170 175
 Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg Leu
 180 185 190
 Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe Arg Cys
 195 200 205
 Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
 210 215 220
 Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
 225 230 235 240
 Ala Asp Cys

<210> 123
 <211> 624
 <212> DNA
 <213> Homo sapiens

<400> 123
 atgaaggagg tggagcagaa ttctggaccc ctcagtgttc cagagggagc cattgcctct 60
 ctcaactgca cttacagtga ccgaggttcc cagtccttct tctggtacag acaatattct 120
 gggaaaagcc ctgagttgat aatgttcata tactccaatg gtgacaaaga agatggaagg 180
 ttacagcac agctcaataa agccagccag tatgtttctc tgctcatcag agactcccag 240
 cccagtgatt cagccaccta cctctgtgcc gtgaaggggg ggtctggggg ttaccagaaa 300
 gttacctttg gaactggaac aaagctccaa gtcaccccaa atatccagaa cccgcatcct 360
 gccgtgtacc agctgagaga ctctaaatcc agtgacaagt ctgtctgcct attcaccgat 420
 tttgattctc aaacaaatgt gtcacaaagt aaggattctg atgtgtatat cacagacaaa 480
 tgtgtgctag acatgaggtc tatggacttc aagagcaaca gtgctgtggc ctggagcaac 540
 aaatctgact ttgcatgtgc aaacgccttc aacaacagca ttattccaga agacaccttc 600

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ttccccagcc cagaaagttc ctaa

624

<210> 124

<211> 735

<212> DNA

<213> Homo sapiens

<400> 124

atggg	cggtca	tgcaga	accc	aagaca	cctg	gtcagg	agga	ggggac	agga	ggcaag	actg	60
agatg	cagcc	caatga	aagg	acacag	tcat	gtttact	ggt	atcggc	cagct	cccagag	gaa	120
ggtct	gaaat	tcatgg	ttta	tctcca	gaaa	gaaaata	tca	tagatg	agtc	aggaat	gcca	180
aagga	acgat	tttctg	ctga	atttccc	aaa	gagggccc	ca	gcatac	ctgag	gatccag	cag	240
gtagt	gcgag	gagatt	cggc	agcttat	tttc	tgtgcc	cagct	caccaca	gac	aggggg	caca	300
gatac	gcagt	attttg	ggcc	aggcac	cccg	ctgacag	tgc	tcgagg	acct	gaaaaa	acgtg	360
ttccc	acccg	aggtcg	ctgt	gtttgag	cca	tcagaag	cag	agatct	ccca	cacccaaa	aag	420
gccac	actgg	tgtgcct	ggc	cacagg	cttc	tacccc	gacc	acgtgg	agct	gagctg	gtg	480
gtgaat	ggga	aggaggt	gca	cagtggg	gtc	tgcacag	acc	cgcagc	ccct	caaggag	cag	540
ccgcgc	ctca	atgact	ccag	atacgt	ctc	agcagc	cgcc	tgagggt	ctc	ggccac	cttc	600
tggcag	gacc	cccgca	acca	cttccg	ctgt	caagtcc	agt	tctacg	ggct	ctcggag	aat	660
gacgag	tgga	cccagg	atag	ggccaa	accc	gtcaccc	aga	tcgtcag	cg	cgaggc	ctgg	720
ggtag	agcag	actaa										735

<210> 125

<211> 207

<212> PRT

<213> Homo sapiens

<400> 125

Met	Lys	Glu	Val	Glu	Gln	Asn	Ser	Gly	Pro	Leu	Ser	Val	Pro	Glu	Gly
1			5					10						15	
Ala	Ile	Ala	Ser	Leu	Asn	Cys	Thr	Tyr	Ser	Asp	Arg	Gly	Ser	Gln	Ser
			20					25					30		
Phe	Phe	Trp	Tyr	Arg	Gln	Tyr	Ser	Gly	Lys	Ser	Pro	Glu	Leu	Ile	Met
		35					40					45			
Phe	Ile	Tyr	Ser	Asn	Gly	Asp	Lys	Glu	Asp	Gly	Arg	Phe	Thr	Ala	Gln
	50					55					60				
Leu	Asn	Lys	Ala	Ser	Gln	Tyr	Val	Ser	Leu	Leu	Ile	Arg	Asp	Ser	Gln
65					70				75						80
Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Lys	Gly	Gly	Ser	Gly
				85					90					95	
Gly	Tyr	Gln	Lys	Val	Thr	Phe	Gly	Thr	Gly	Thr	Lys	Leu	Gln	Val	Ile
			100					105					110		
Pro	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser
		115					120					125			
Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln
			130				135				140				
Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys
145					150					155					160

Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val
165 170 175

<400> 126

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Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp
 225 230 235 240

Gly Arg Ala Asp

<210> 127
 <211> 621
 <212> DNA
 <213> Homo sapiens

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 tctgggaaaa gccctgagtt gataatgtcc atatactcca atggtgacaa agaagatgga 180
 aggtttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
 cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg 300
 cagtttggag cagggaccca ggttgtggtc accccagata tccagaacct tgacctgcc 360
 gtgtaccagc tgagagactc taaatccagt gacaagtctg tctgcctatt caccgatttt 420
 gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaatgt 480
 gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
 tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc 600
 cccagcccag aaagttccta a 621

<210> 128
 <211> 206
 <212> PRT
 <213> Homo sapiens

<400> 128
 Met Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu
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 Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln
 20 25 30
 Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45
 Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60
 Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80
 Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95
 Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110
 Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 115 120 125
 Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140

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Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
 145 150 155 160

Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 129

<211> 621

<212> DNA

<213> Homo sapiens

<400> 129

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tctgggaaaa gccctgagtt gataatgtcc atatactcca atggtgacaa agaagatgga      180
aggtttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc      240
cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg      300
cagtttggag cagggaccca ggttggtggtc accccagata tccagaaccc tgaccctgcc      360
gtgtaccagc tgagagactc taaatccagt gacaagtctg tctgcctatt caccgatttt      420
gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatctg tgacaaaact      480
gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa      540
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cccgcccag aaagttccta a                                     621

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<210> 130

<211> 206

<212> PRT

<213> Homo sapiens

<400> 130

Met Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu
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 20 25 30

Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45

Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60

Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80

Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95

Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110

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Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 115 120 125

Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Cys Asp Lys Thr
 145 150 155 160

Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 131
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 <212> DNA
 <213> Homo sapiens

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 aggttttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
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 gtgtaccagc tgagagactc taaatccagt gacaagtctg tctgcctatt caccgatttt 420
 gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaaact 480
 gtgctagaca tgaggtctat ggacttcaag agcaactgtg ctgtggcctg gagcaacaaa 540
 tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc 600
 cccagcccag aaagttccta a 621

<210> 132
 <211> 206
 <212> PRT
 <213> Homo sapiens

<400> 132
 Met Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu
 1 5 10 15
 Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln
 20 25 30
 Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45
 Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60
 Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80

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Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95

Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110

Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 115 120 125

Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
 145 150 155 160

Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Cys Ala Val Ala
 165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 133
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 <213> Homo sapiens

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 aggttttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
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 gtgtgtgaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
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 ccagcccag aaagttccta a 621

<210> 134
 <211> 206
 <212> PRT
 <213> Homo sapiens

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 20 25 30

Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45

-115-

Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
50 55 60

Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
65 70 75 80

Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
85 90 95

Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
100 105 110

Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
115 120 125

Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
130 135 140

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
145 150 155 160

Val Cys Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
195 200 205

<210> 135
<211> 621
<212> DNA
<213> Homo sapiens

<400> 135
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aggttttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg 300
cagttttggag cagggaccca gggtgtggtc accccagata tccagaaccc tgaccctgcc 360
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gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaaact 480
gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc 600
cccagcccag aaagttccta a 621

<210> 136
<211> 206
<212> PRT
<213> Homo sapiens

<400> 136
Met Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu
1 5 10 15

-116-

Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln
 20 25 30
 Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45
 Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60
 Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80
 Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95
 Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110
 Asp Ile Gln Asn Pro Asp Pro Ala Val Cys Gln Leu Arg Asp Ser Lys
 115 120 125
 Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140
 Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
 145 150 155 160
 Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175
 Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190
 Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 137

<211> 621

<212> DNA

<213> Homo sapiens

<400> 137

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tctgggaaaa gccctgagtt gataatgtcc atatactcca atggtgacaa agaagatgga      180
aggttttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc      240
cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg      300
cagtttggag cagggaccca ggttgtggtc accccagata tccagaaccc tgaccctgcc      360
gtgtaccagc tgagagactg taaatccagt gacaagtctg tctgcctatt caccgatttt      420
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gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa      540
tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc      600
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<210> 138

<211> 206

<212> PRT

<213> Homo sapiens

-117-

<400> 138

Met Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu
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Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln
 20 25 30

Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45

Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60

Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80

Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95

Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110

Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Cys Lys
 115 120 125

Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
 145 150 155 160

Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 139

<211> 621

<212> DNA

<213> Homo sapiens

<400> 139

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 tctgggaaaa gccctgagtt gataatgtcc atatactcca atgggtgacaa agaagatgga 180
 aggttttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
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 gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
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 cccagcccag aaagttccta a 621

-118-

<210> 140
 <211> 206
 <212> PRT
 <213> Homo sapiens

<400> 140
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 Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln
 20 25 30
 Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45
 Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60
 Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80
 Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95
 Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110
 Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Cys Arg Asp Ser Lys
 115 120 125
 Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140
 Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
 145 150 155 160
 Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175
 Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190
 Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 141
 <211> 621
 <212> DNA
 <213> Homo sapiens

<400> 141
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 cagcccagtg attcagccac ctacctctgt gccgttacaa ctgacagctg ggggaaattg 300
 cagtttggag cagggaccca ggttggtggtc accccagata tccagaaccc tgaccctgcc 360

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gtgtaccagc tgagagactc taaatccagt gacaagtctt gctgcctatt caccgatttt 420
gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaaact 480
gtgctagaca tgaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
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cccagcccag aaagttccta a 621

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<210> 142
<211> 206
<212> PRT
<213> Homo sapiens

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<400> 142
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20 25 30

Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
35 40 45

Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
50 55 60

Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
65 70 75 80

Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
85 90 95

Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
100 105 110

Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
115 120 125

Ser Ser Asp Lys Ser Cys Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
130 135 140

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
145 150 155 160

Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
165 170 175

Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
180 185 190

Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
195 200 205

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<210> 143
<211> 621
<212> DNA
<213> Homo sapiens

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<400> 143
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aggtttacag cacagctcaa taaagccagc cagtatgttt ctctgctcat cagagactcc 240
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gattctcaaa caaatgtgtc acaaagtaag gattctgatg tgtatatcac agacaaaact 480
gtgctagact gtaggtctat ggacttcaag agcaacagtg ctgtggcctg gagcaacaaa 540
tctgactttg catgtgcaaa cgccttcaac aacagcatta ttccagaaga caccttcttc 600
cccagcccag aaagttccta a 621

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<210> 144
 <211> 206
 <212> PRT
 <213> Homo sapiens

<400> 144
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 20 25 30
 Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile
 35 40 45
 Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala
 50 55 60
 Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser
 65 70 75 80
 Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Thr Thr Asp Ser
 85 90 95
 Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr Pro
 100 105 110
 Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 115 120 125
 Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
 130 135 140
 Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr
 145 150 155 160
 Val Leu Asp Cys Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
 165 170 175
 Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
 180 185 190
 Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 145
 <211> 621

-121-

<212> DNA

<213> Homo sapiens

<400> 145

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tctgggaaaa	gccctgagtt	gataatgtcc	atatactcca	atggtgacaa	agaagatgga	180
aggtttacag	cacagctcaa	taaagccagc	cagtatgttt	ctctgctcat	cagagactcc	240
cagcccagtg	attcagccac	ctacctctgt	gccgttacaa	ctgacagctg	ggggaaattg	300
cagtttggag	cagggaccca	ggttggtggtc	accccagata	tccagaaccc	tgaccctgcc	360
gtgtaccagc	tgagagactc	taaatccagt	gacaagtctg	tctgcctatt	caccgatttt	420
gattctcaaa	caaatgtgtc	acaaagtaag	gattctgatg	tgtgtatcac	agacaaaact	480
gtgctagaca	tgaggtctat	ggacttcaag	agcaacagtg	ctgtggcctg	gagcaacaaa	540
tctgactttg	catgtgcaaa	cgccttcaac	aacagcatta	ttccagaaga	caccttcttc	600
cccagcccag	aaagttccta	a				621

<210> 146

<211> 206

<212> PRT

<213> Homo sapiens

<400> 146

Met	Gln	Lys	Glu	Val	Glu	Gln	Asn	Ser	Gly	Pro	Leu	Ser	Val	Pro	Glu
1				5					10					15	
Gly	Ala	Ile	Ala	Ser	Leu	Asn	Cys	Thr	Tyr	Ser	Asp	Arg	Gly	Ser	Gln
			20					25					30		
Ser	Phe	Phe	Trp	Tyr	Arg	Gln	Tyr	Ser	Gly	Lys	Ser	Pro	Glu	Leu	Ile
		35					40					45			
Met	Ser	Ile	Tyr	Ser	Asn	Gly	Asp	Lys	Glu	Asp	Gly	Arg	Phe	Thr	Ala
	50					55					60				
Gln	Leu	Asn	Lys	Ala	Ser	Gln	Tyr	Val	Ser	Leu	Leu	Ile	Arg	Asp	Ser
65					70					75					80
Gln	Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Thr	Thr	Asp	Ser
				85					90					95	
Trp	Gly	Lys	Leu	Gln	Phe	Gly	Ala	Gly	Thr	Gln	Val	Val	Val	Thr	Pro
			100					105						110	
Asp	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys
		115					120					125			
Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr
	130					135					140				
Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Cys	Ile	Thr	Asp	Lys	Thr
145					150					155					160
Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala
				165					170					175	
Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser
			180					185					190		

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Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser
 195 200 205

<210> 147
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 147
 atgaacgctg gtgtcactca gacccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
 aactgcagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcatggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcc a gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cgggcccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc cacccgaggt cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tggtgggtga atgggaagga ggtgcacagt ggggtctgca cagacccgca gccctcaag 540
 gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 148
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 148
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60
 Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80
 Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160

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Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro
 165 170 175
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240
 Ala Trp Gly Arg Ala Asp
 245

<210> 149
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 149
 atgaacgctg gtgtcactca gaccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
 aactgcaagt gtgccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcatggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cgggccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc caccgaggt cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tgggtgggtga atgggaagga ggtgcacagt ggggtcagca cagaccgca gccctcaag 540
 gagcagcccg cctcaatga ctccagatac gctctgtgta gccgctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggacca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 150
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 150
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60

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Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80
 Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
 165 170 175
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Cys Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240
 Ala Trp Gly Arg Ala Asp
 245

<210> 151
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 151
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 aactgacagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcattggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cgggcccgggc accaggctca cggtcacaga ggacctgaaa 360
 aacgtgttcc caccgaggt cgctgtgttt gagccatgtg aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tgggtgggtga atgggaagga ggtgcacagt ggggtcagca cagaccgcga gccctcaag 540
 gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaaccogtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 152
 <211> 246

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<212> PRT

<213> Homo sapiens

<400> 152

Met	Asn	Ala	Gly	Val	Thr	Gln	Thr	Pro	Lys	Phe	Gln	Val	Leu	Lys	Thr
1				5					10					15	

Gly	Gln	Ser	Met	Thr	Leu	Gln	Cys	Ala	Gln	Asp	Met	Asn	His	Glu	Tyr
			20					25					30		

Met	Ser	Trp	Tyr	Arg	Gln	Asp	Pro	Gly	Met	Gly	Leu	Arg	Leu	Ile	His
		35					40					45			

Tyr	Ser	Val	Gly	Ala	Gly	Ile	Thr	Asp	Gln	Gly	Glu	Val	Pro	Asn	Gly
	50					55					60				

Tyr	Asn	Val	Ser	Arg	Ser	Thr	Thr	Glu	Asp	Phe	Pro	Leu	Arg	Leu	Leu
65					70					75					80

Ser	Ala	Ala	Pro	Ser	Gln	Thr	Ser	Val	Tyr	Phe	Cys	Ala	Ser	Arg	Pro
				85					90					95	

Gly	Leu	Ala	Gly	Gly	Arg	Pro	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg
			100					105					110		

Leu	Thr	Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala
		115					120					125			

Val	Phe	Glu	Pro	Cys	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr
	130					135					140				

Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser
145					150					155					160

Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Ser	Thr	Asp	Pro
				165					170					175	

Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Ala	Leu
			180					185						190	

Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asp	Pro	Arg	Asn
		195					200					205			

His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu
	210					215					220				

Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu
225					230					235					240

Ala	Trp	Gly	Arg	Ala	Asp
				245	

<210> 153

<211> 741

<212> DNA

<213> Homo sapiens

<400> 153

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atgaacgctg gtgtcactca gacccccaaaa ttccagggtcc tgaagacagg acagagcatg      60
acactgcagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca      120
ggcatggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa      180
gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg      240
tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga      300
gggcgaccag agcagtactt cggggccgggc accagggtca cggtcacaga ggacctgaaa      360
aacgtgttcc cacccgaggt cgcttggttt gagccatcag aagcagagat ctcccacacc      420
caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc      480
tggtgggtga atgggaagga ggtgcacagt ggggtcagca cagacccgca gcccctcaag      540
gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc      600
accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg      660
gagaatgacg agtggaccca ggataggggc aaaccctgca cccagatcgt cagcgccgag      720
gcctggggta gagcagacta a                                741

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<210> 154
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 154
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60
 Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80
 Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Cys Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
 165 170 175
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205

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His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
210 215 220

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
225 230 235 240

Ala Trp Gly Arg Ala Asp
245

<210> 155
<211> 741
<212> DNA
<213> Homo sapiens

<400> 155
atgaacgctg gtgtcactca gacccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
acactgcagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
ggcatggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
tcggctgctc cctcccagac atctgtgtac ttctgtgcc a gcaggccggg actagcggga 300
gggcgaccag agcagtactt cgggcccgggc accaggctca cggtcacaga ggacctgaaa 360
aacgtgttcc cacccgaggc cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
tggtgggtga atgggaagga ggtgcacagt ggggtcagca catgcccgca gccctcaag 540
gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
gagaatgacg agtggaccca ggatagggcc aaaccctgca cccagatcgt cagcgccgag 720
gcctggggta gagcagacta a 741

<210> 156
<211> 246
<212> PRT
<213> Homo sapiens

<400> 156
Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
1 5 10 15
Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
20 25 30
Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
35 40 45
Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
50 55 60
Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
65 70 75 80
Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
85 90 95
Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
100 105 110

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Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Cys Pro
 165 170 175
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240
 Ala Trp Gly Arg Ala Asp
 245

<210> 157
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 157
 atgaacgctg gtgtcactca gaccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
 aactgcagt gtgccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcatggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgccg gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cggggccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc cacccgaggt cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc cggaccacgt ggagctgagc 480
 tggtgggtga atgggaagga ggtgcacagt ggggtcagca cagaccgcga gccctcaag 540
 gagcagcccg cctcaatga ctccagatac gctctgagca gctgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggacca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 158
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 158
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30

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Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
35 40 45

Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
50 55 60

Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
65 70 75 80

Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
85 90 95

Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
100 105 110

Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
115 120 125

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
130 135 140

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
145 150 155 160

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
165 170 175

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
180 185 190

Ser Ser Cys Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
195 200 205

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
210 215 220

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
225 230 235 240

Ala Trp Gly Arg Ala Asp
245

<210> 159

<211> 741

<212> DNA

<213> Homo sapiens

<400> 159

atgaacgctg	gtgtcactca	gacccccaaa	ttccaggtcc	tgaagacagg	acagagcatg	60
acactgcagt	gtgcccagga	tatgaaccat	gaatacatgt	cctggtatcg	acaagaccca	120
ggcatggggc	tgaggctgat	tcattactca	gttggtgctg	gtatcactga	ccaaggagaa	180
gtccccaatg	gctacaatgt	ctccagatca	accacagagg	atttcccgct	caggctgctg	240
tcggctgctc	cctcccagac	atctgtgtac	ttctgtgcca	gcaggccggg	actagcggga	300
gggcgaccag	agcagtactt	cgggccgggc	accaggctca	cggtcacaga	ggacctgaaa	360
aacgtgttcc	cacccgaggt	cgctgtgtgt	gagccatcag	aagcagagat	ctccacacc	420
caaaaggcca	cactggtgtg	cctggccaca	ggcttctacc	ccgaccacgt	ggagctgagc	480
tggtgggtga	atgggaagga	ggtgcacagt	ggggtcagca	cagacccgca	gccctcaag	540

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gagcagccccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 160
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 160
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60
 Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80
 Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Cys Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
 165 170 175
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240
 Ala Trp Gly Arg Ala Asp
 245

-131-

<210> 161
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 161
 atgaacgctg gtgtcactca gacccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
 acactgcagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcattggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cggggccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc cacccgaggt cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactgggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tggtgggtga atgggaagga ggtgcacagt tgtgtcagca cagacccgca gcccctcaag 540
 gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 162
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 162
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60
 Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80
 Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160

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Trp Trp Val Asn Gly Lys Glu Val His Ser Cys Val Ser Thr Asp Pro
 165 170 175

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240

Ala Trp Gly Arg Ala Asp
 245

<210> 163
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 163
 atgaacgctg gtgtcactca gacccccaaaa ttccagggtcc tgaagacagg acagagcatg 60
 acactgcagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcattggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cggggccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc cacccgaggt cgctgtgttt gagccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tggtgggtga atgggaagga ggtgcacagt ggggtcagca cagacccgca gccctgcaag 540
 gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 164
 <211> 246
 <212> PRT
 <213> Homo sapiens

<400> 164
 Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
 1 5 10 15
 Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
 20 25 30
 Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
 35 40 45
 Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly
 50 55 60
 Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu
 65 70 75 80

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Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Arg Pro
 85 90 95
 Gly Leu Ala Gly Gly Arg Pro Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 100 105 110
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 115 120 125
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 130 135 140
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 145 150 155 160
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
 165 170 175
 Gln Pro Cys Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu
 180 185 190
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn
 195 200 205
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 210 215 220
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 225 230 235 240
 Ala Trp Gly Arg Ala Asp
 245

<210> 165
 <211> 741
 <212> DNA
 <213> Homo sapiens

<400> 165
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 aactgacagt gtgcccagga tatgaaccat gaatacatgt cctgggtatcg acaagaccca 120
 ggcattggggc tgaggctgat tcattactca gttgggtgctg gtatcactga ccaaggagaa 180
 gtccccaatg gctacaatgt ctccagatca accacagagg atttcccgtc caggctgctg 240
 tcggctgctc cctcccagac atctgtgtac ttctgtgcca gcaggccggg actagcggga 300
 gggcgaccag agcagtactt cgggcccgggc accagggtca cggtcacaga ggacctgaaa 360
 aacgtgttcc caccagaggt cgctgtgttt tgtccatcag aagcagagat ctcccacacc 420
 caaaaggcca cactggtgtg cctggccaca ggcttctacc ccgaccacgt ggagctgagc 480
 tggtgggtga atgggaagga ggtgcacagt ggggtcagca cagacccgca gccctcaag 540
 gagcagcccg ccctcaatga ctccagatac gctctgagca gccgcctgag ggtctcggcc 600
 accttctggc aggacccccg caaccacttc cgctgtcaag tccagttcta cgggctctcg 660
 gagaatgacg agtggaccca ggatagggcc aaacccgtca cccagatcgt cagcgccgag 720
 gcctggggta gagcagacta a 741

<210> 166
 <211> 246
 <212> PRT
 <213> Homo sapiens

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<400> 166

Met	Asn	Ala	Gly	Val	Thr	Gln	Thr	Pro	Lys	Phe	Gln	Val	Leu	Lys	Thr
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Gly	Gln	Ser	Met	Thr	Leu	Gln	Cys	Ala	Gln	Asp	Met	Asn	His	Glu	Tyr
			20					25					30		

Met	Ser	Trp	Tyr	Arg	Gln	Asp	Pro	Gly	Met	Gly	Leu	Arg	Leu	Ile	His
		35					40					45			

Tyr	Ser	Val	Gly	Ala	Gly	Ile	Thr	Asp	Gln	Gly	Glu	Val	Pro	Asn	Gly
	50					55					60				

Tyr	Asn	Val	Ser	Arg	Ser	Thr	Thr	Glu	Asp	Phe	Pro	Leu	Arg	Leu	Leu
65					70					75					80

Ser	Ala	Ala	Pro	Ser	Gln	Thr	Ser	Val	Tyr	Phe	Cys	Ala	Ser	Arg	Pro
				85					90					95	

Gly	Leu	Ala	Gly	Gly	Arg	Pro	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg
			100					105					110		

Leu	Thr	Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala
		115					120					125			

Val	Phe	Cys	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr
	130					135					140				

Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser
145					150					155					160

Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Ser	Thr	Asp	Pro
				165					170					175	

Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Ala	Leu
			180					185					190		

Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asp	Pro	Arg	Asn
		195					200					205			

His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu
	210					215					220				

Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu
225					230					235					240

Ala	Trp	Gly	Arg	Ala	Asp
					245

<210> 167

<211> 783

<212> DNA

<213> Homo sapiens

<400> 167

atgggtgtca	ctcagacccc	aaaattccag	gtcctgaaga	caggacagag	catgacactg	60
cagtgtgccc	aggatatgaa	ccatgaatac	atgtcctggt	atcgacaaga	cccaggcatg	120

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gggctgaggc tgattcatta ctcagttggt gctggtatca ctgaccaagg agaagtcccc 180
aatggctaca atgtctccag atcaaccaca gaggatttcc cgctcaggct gctgtcggct 240
gctccctccc agacatctgt gtacttctgt gccagcagtt acgtcgggaa caccggggag 300
ctgttttttg gagaaggctc taggctgacc gtactggagg acctgaaaaa cgtgttccca 360
cccgaggctc ctgtgtttga gccatcagaa gcagagatct cccacacca aaaggccaca 420
ctgggtgtgcc tggccacagg cttctacccc gaccacgtgg agctgagctg gtgggtgaat 480
gggaaggagg tgcacagtgg ggtctgcaca gaccgcgagc ccctcaagga gcagcccgcc 540
ctcaatgact ccagatacgc tctgagcagc cgcctgaggg tctcggccac cttctggcag 600
gacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag 660
tggacccagg atagggccaa acccgtcacc cagatcgtca gcgccgaggc ctggggtaga 720
gcagacggat ccggtggtgg tctgaacgat atttttgaag ctcagaaaat cgaatggcat 780
taa 783

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<210> 168
 <211> 260
 <212> PRT
 <213> Homo sapiens

<400> 168
 Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
 1 5 10 15
 Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
 20 25 30
 Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
 35 40 45
 Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
 50 55 60
 Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
 65 70 75 80
 Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
 85 90 95
 Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
 100 105 110
 Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
 115 120 125
 Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
 130 135 140
 Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
 145 150 155 160
 Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
 165 170 175
 Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg Leu
 180 185 190
 Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe Arg Cys
 195 200 205

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Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
 210 215 220

Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
 225 230 235 240

Ala Asp Gly Ser Gly Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys
 245 250 255

Ile Glu Trp His
 260

<210> 169
 <211> 762
 <212> DNA
 <213> Homo sapiens

<400> 169
 atgggtgtca ctcagacccc aaaattccag gtcctgaaga caggacagag catgacactg 60
 cagtgtgccc aggatatgaa ccatgaatac atgtcctggg atcgacaaga cccaggcatg 120
 gggctgaggg tgattcatta ctcagttggg gctgggtatca ctgaccaagg agaagtcccc 180
 aatggctaca atgtctccag atcaaccaca gaggatttcc cgctcagggt gctgtcgggt 240
 gctccctccc agacatctgt gtacttctgt gccagcagtt acgtcgggaa caccggggag 300
 ctgttttttg gagaaggctc taggctgacc gtactggagg acctgaaaaa cgtgttccca 360
 cccgaggteg ctgtgtttga gccatcagaa gcagagatct cccacacca aaaggccaca 420
 ctgggtgtgcc tggccacagg cttctacccc gaccacgtgg agctgagctg gtgggtgaat 480
 gggaaggagg tgcacagtgg ggtctgcaca gaccgcagc ccctcaagga gcagcccgcc 540
 ctcaatgact ccagatacgc tctgagcagc cgcctgaggg tctcggccac cttctggcag 600
 gacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag 660
 tggacccagg atagggccaa acccgtcacc cagatcgtca gcgccgaggg ctgggggtaga 720
 gcagacggat ccggtggtgg tcatcatcac catcatcact aa 762

<210> 170
 <211> 253
 <212> PRT
 <213> Homo sapiens

<400> 170
 Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
 1 5 10 15
 Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
 20 25 30
 Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
 35 40 45
 Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
 50 55 60
 Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
 65 70 75 80
 Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
 85 90 95
 Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
 100 105 110

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Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
 115 120 125

Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
 130 135 140

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
 145 150 155 160

Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
 165 170 175

Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg Leu
 180 185 190

Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe Arg Cys
 195 200 205

Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
 210 215 220

Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
 225 230 235 240

Ala Asp Gly Ser Gly Gly Gly His His His His His His
 245 250

<210> 171

<211> 741

<212> DNA

<213> Homo sapiens

<400> 171

atgaacgctg	gtgtcactca	gacccccaaaa	ttccaggtcc	tgaagacagg	acagagcatg	60
acactgcagt	gtgcccagga	tatgaaccat	gaatacatgt	cctgggtatcg	acaagaccca	120
ggcatggggc	tgaggctgat	tcattactca	gttggtgctg	gtatcactga	ccaaggagaa	180
gtccccaatg	gctacaatgt	ctccagatca	accacagagg	atttcccgct	caggctgctg	240
tcggctgctc	cctcccagac	atctgtgtac	ttctgtgcca	gcaggccggg	actagcggga	300
gggcgaccag	agcagtactt	cgggccgggc	accaggctca	cggtcacaga	ggacctgaac	360
aaggtgttcc	cacccgaggt	cgctgtgttt	gagccatcag	aagcagagat	ctcccacacc	420
caaaaggcca	cactggtgtg	cctggccaca	ggcttcttcc	ccgaccacgt	ggagctgagc	480
tggtgggtga	atgggaagga	ggtgcacagt	gggtctgca	cagacccgca	gcccctcaag	540
gagcagcccc	ccctcaatga	ctccagatac	tctctgagca	gccgcctgag	ggtctcggcc	600
accttctggc	aggacccccg	caaccacttc	cgctgtcaag	tccagttcta	cgggctctcg	660
gagaatgacg	agtggaccca	ggatagggcc	aaacccgtca	cccagatcgt	cagcgccgag	720
gcctggggta	gagcagacta	a				741

<210> 172

<211> 741

<212> DNA

<213> Homo sapiens

<400> 172

atgaacgctg	gtgtcactca	gacccccaaaa	ttccaggtcc	tgaagacagg	acagagcatg	60
acactgcagt	gtgcccagga	tatgaaccat	gaatacatgt	cctgggtatcg	acaagaccca	120
ggcatggggc	tgaggctgat	tcattactca	gttggtgctg	gtatcactga	ccaaggagaa	180
gtccccaatg	gctacaatgt	ctccagatca	accacagagg	atttcccgct	caggctgctg	240

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tcggctgctc	cctcccagac	atctgtgtac	ttctgtgcca	gcaggccggg	actagcggga	300
gggcgaccag	agcagtactt	cgggccgggc	accaggctca	cggtcacaga	ggacctgaaa	360
aacgtgttcc	cacccgaggt	cgtgtgtgtt	gagccatcag	aagcagagat	ctcccacacc	420
caaaaggcca	cactggtgtg	cctggccaca	ggcttctacc	ccgaccacgt	ggagctgagc	480
tggtgggtga	atgggaagga	ggtgcacagt	gggtcttgca	cagacccgca	gcccctcaag	540
gagcagcccg	ccctcaatga	ctccagatac	tgtctgagca	gccgcctgag	ggtctcggcc	600
accttctggc	aggacccccg	caaccacttc	cgtgttcaag	tccagttcta	cgggctctcg	660
gagaatgacg	agtggaccca	ggatagggcc	aaaccctgca	cccagatcgt	cagcgccgag	720
gcctggggta	gagcagacta	a				741

<210> 173

<211> 741

<212> DNA

<213> Homo sapiens

<400> 173

atgaacgctg	gtgtcactca	gacccccaaa	ttccaggctc	tgaagacagg	acagagcatg	60
acactgcagt	gtgcccagga	tatgaaccat	gaatacatgt	cctgggtatcg	acaagaccca	120
ggcatggggc	tgaggctgat	tcattactca	gttggtgctg	gtatcactga	ccaaggagaa	180
gtccccaatg	gctacaatgt	ctccagatca	accacagagg	atttcccgtc	caggctgctg	240
tcggctgctc	cctcccagac	atctgtgtac	ttctgtgcca	gcaggccggg	actagcggga	300
gggcgaccag	agcagtactt	cgggccgggc	accaggctca	cggtcacaga	ggacctgaaa	360
aacgtgttcc	cacccgaggt	cgtgtgtgtt	gagccatcag	aagcagagat	ctcccacacc	420
caaaaggcca	cactggtgtg	cctggccaca	ggcttctacc	ccgaccacgt	ggagctgagc	480
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gagaatgacg	agtggaccca	ggatagggcc	aaaccctgca	cccagatcgt	cagcgccgag	720
gcctggggta	gagcagacta	a				741

<210> 174

<211> 6671

<212> DNA

<213> Expression vector pYX112

<400> 174

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gtgcctgact	acgcatgata	tctcgagctc	agctagctaa	ctgaataagg	aacaatgaac	120
gttttttcc	tctcttggtc	ctagtattaa	tgactgaccg	atacatccct	tttttttttt	180
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taaatcgggg	gctcccttta	gggttcogat	ttagtggttt	acggcacctc	gacccccaaa	600
aacttgatta	gggtgatggt	tcacgtagt	ggccatcgcc	ctgatagacg	gttttttcgcc	660
ctttgacgtt	ggagtccacg	ttctttaata	gtggactctt	gttccaaact	ggaacaacac	720
tcaaccctat	ctcgggtctat	tcttttgatt	tataagggat	tttgccgatt	tcggcctatt	780
ggttaaaaaa	tgagctgatt	taacaaaaat	ttaacgcgaa	ttttaacaaa	atattaacgt	840
ttacaatttc	ctgatgcggt	attttctcct	tacgcactct	tgcggtat	cacaccgcat	900
agggtataaa	ctgatataat	taaattgaag	ctctaatttg	tgagtttagt	atacatgcat	960
ttacttataa	tacagttttt	tagttttgct	ggccgcactc	tctcaaata	gcttcccagc	1020
ctgcttttct	gtaacgttca	ccctgtacct	tagcatccct	tccctttgca	aatagtcctc	1080
ttccaacaat	aataatgtca	gacccgtgag	agaccacatc	atccacggtt	ctatactggt	1140
gacccaatgc	gtctcccttg	tcactctaac	ccacaccggg	tgtcataatc	aaccaatcgt	1200
aaccttcac	tcttccaccc	atgtctcttt	gagcaataaa	gccgataaca	aaatctttgt	1260
cgtcttctgc	aatgtcaaca	gtacccttag	tataattctc	agtagatagg	gagcccttgc	1320

atgacaattc	tgctaacatc	aaaaggcctc	taggttcctt	tgttacttct	tctgccgcct	1380
gcttcaaacc	gctaacaata	cctggcccca	gcacaccgtg	tgcattegtg	atgtctgccc	1440
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caaattttct	gtcttcgaag	agtaaaaaat	tgtacttggc	ggataatgcc	tttagcggct	1560
taactgtgcc	ctccatcgaa	aaatcagtca	atataatccac	atgtgttttt	agtaaacaaa	1620
ttttgggacc	taatgcttca	actaactcca	gtaattcctt	ggtggtacga	acatccaatg	1680
aagcacacaa	gtttgtttgc	ttttcgtgca	tgatattaaa	tagcttggca	gcaacaggac	1740
taggatgagt	agcagcacgt	tccttatatg	tagctttcga	catgatttat	cttcgtttcc	1800
tgacaggttt	tgcttctgtc	agttgggtta	agaatactgg	gcaatttcat	gtttcttcaa	1860
cactacatat	gcgtatatat	accaatctaa	gtctgtgctc	cttccttcgt	tcttccttct	1920
gttcggagat	taccgaatca	aaaaaatttc	aaagaaaccg	aaatcaaaaa	aaagaataaaa	1980
aaaaaaatga	tgaattgaat	tgaaaagctg	tggtatgggtg	cactctcagt	acaatctgct	2040
ctgatgccgc	atagttaagc	cagccccgac	acccgccaac	acccgctgac	gcgccctgac	2100
gggcttgtct	gctcccggca	tcgccttaca	gacaagctgt	gaccgtctcc	gggagctgca	2160
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gcctatthtt	ataggthaat	gtcatgataa	taatggthtc	ttaggacgga	tcgcttgccct	2280
gtaacttaca	cgcgcctcgt	atctthttaat	gatggaataa	tttgggaatt	tactctgtgt	2340
ttatthattt	ttatgtthtg	tatthggatt	ttagaaagta	aataaagaag	gtagaagagt	2400
tacggaatga	agaaaaaaa	ataaacaag	gtthaaaaa	tttcaacaaa	aagcgtactt	2460
tacatatata	tttattagac	aagaaaagca	gattaaatag	atatacatte	gattaacgat	2520
aagtaaaatg	taaaatcaca	ggatthtcgt	gtgtggthct	ctacacagac	aagatgaaac	2580
aattcggcat	taataacctga	gagcaggaag	agcaagataa	aaggtagtat	ttgttggcga	2640
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Claims

1. A soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, characterised in that (i) and (ii) are linked by a disulphide bond between cysteine residues substituted for:

Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01;
 Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01;
 Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01;
 Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01; or
 Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01.
2. A sTCR as claimed in claim 1, wherein one or both of (i) and (ii) comprise all of an extracellular constant Ig domain of the TCR chain.
3. A sTCR as claimed in claim 1 or claim 2, wherein one or both of (i) and (ii) comprise all of an extracellular domain of the TCR chain.
4. A soluble $\alpha\beta$ -form T cell receptor (sTCR), wherein a covalent disulphide bond links cysteine residues substituted for:

Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01;
 Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01;
 Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01;
 Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01; or
 Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01.
5. A soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain, except the transmembrane domain thereof, and (ii) all or part of a TCR β chain, except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable

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domain and at least a part of the constant domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR and wherein an interchain disulphide bond in native TCR is not present.

5 6. A sTCR as claimed in claim 5, wherein one or both of (i) and (ii) comprise all of an extracellular constant Ig domain of the TCR chain.

7. A sTCR as claimed in claim 5 or claim 6, wherein one or both of (i) and (ii) comprise all of an extracellular domain of the TCR chain.

10

8. A soluble $\alpha\beta$ -form T cell receptor (sTCR), wherein a covalent disulphide bond links a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain, wherein an interchain disulphide bond in native TCR is not present.

15

9. A sTCR as claimed in any one of claims 5 to 8, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for residues whose β carbon atoms are less than 0.6 nm apart in the native TCR structure.

20 10. A sTCR as claimed in any one of claims 5 to 9, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01.

11. A sTCR as claimed in any one of claims 5 to 9, wherein the disulphide bond which is
25 not present in native TCR is between cysteine residues substituted for Thr 45 of exon 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01.

12. A sTCR as claimed in any one of claims 5 to 9, wherein the disulphide bond which is
30 not present in native TCR is between cysteine residues substituted for Tyr 10 of exon 1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01.

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13. A sTCR as claimed in any one of claims 5 to 9, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for Thr 45 of exon 1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01.

5

14. A sTCR as claimed in any one of claims 5 to 9, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01.

10 15. A sTCR as claimed in any one of claims 1 to 4, wherein an interchain disulphide bond in native TCR is not present.

15 16. A sTCR as claimed in any one of claims 5 to 15, wherein native α and β TCR chains are truncated at the C-terminus such that the cysteine residues which form the native interchain disulphide bond are excluded.

17. A sTCR as claimed in any one of claims 5 to 15, wherein cysteine residues which form the native interchain disulphide bond are substituted to another residue.

20 18. A sTCR as claimed in claim 17, wherein cysteine residues which form the native interchain disulphide bond are substituted to serine or alanine.

19. A sTCR as claimed in any one of claims 1 to 18, wherein an unpaired cysteine residue present in native TCR β chain is not present.

25

20. A sTCR as claimed in any one of claims 1, 2, 5, 6 and 9 to 19, wherein (i) and (ii) each comprise the functional variable domain of a first TCR fused to all or part of the constant domain of a second TCR, the first and second TCRs being from the same species.

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21. A sTCR as claimed in claim 20, wherein the constant domains of the second TCR are truncated N-terminal to the residues which form the non-native interchain disulphide bond.

22. A sTCR as claimed in any one of claims 1 to 21, wherein one or both of the chains are derivatised with, or fused to, a moiety at its C or N terminus.

23. A sTCR as claimed in any one of claims 1 to 22, wherein one or both of the chains have a cysteine residue at its C terminus, its N terminus or both termini, to which a moiety can be fused.

10

24. A sTCR as claimed in any one of claims 1 to 23 further comprising a detectable label.

25. A sTCR as claimed in any one of claims 1 to 24 associated with a therapeutic agent.

15 26. A multivalent T cell receptor (TCR) complex comprising a plurality of sTCRs as claimed in any one of claims 1 to 25.

27. A complex as claimed in claim 26, comprising a sTCR multimer.

20 28. A complex as claimed in claim 27, comprising two or three or four or more T cell receptor molecules associated with one another.

29. The complex of claim 28, wherein two or more of the T cell receptor molecules are associated via a linker molecule.

25

30. A complex as claimed in any one of claims 26 to 29, wherein the sTCRs or sTCR multimers are present in a lipid bilayer or are attached to a particle.

31. A method for detecting MHC-peptide complexes, which comprises:

30 (i) providing a soluble TCR as claimed in any one of claims 1 to 25 or a multivalent T cell receptor complex as claimed in any one of claims 26 to 30;

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(ii) contacting the soluble TCR or multivalent TCR complex with the MHC-peptide complexes; and

(iii) detecting binding of the soluble TCR or multivalent TCR complex to the MHC-peptide complexes.

5

32. A pharmaceutical formulation comprising a sTCR as claimed in any one of claims 1 to 25, a multivalent TCR complex as claimed in any one of claims 26 to 30, or both of said sTCR and said multivalent TCR complex, together with a pharmaceutically acceptable carrier.

10 33. A nucleic acid molecule comprising a sequence encoding (i) or (ii) of a sTCR as defined in any one of claims 1 to 25, or a sequence complementary thereto.

34. A vector comprising a nucleic acid molecule as claimed in claim 33.

15 35. A host cell comprising a vector as claimed in claim 34.

36. A method for obtaining (i) or (ii) as defined in any one of claims 1 to 25, which method comprises incubating a host cell as claimed in claim 35 under conditions causing expression of the peptide and then purifying the polypeptide.

20

37. A method as claimed in claim 36, further comprising mixing (i) and (ii) under suitable refolding conditions.

38. A method for obtaining a soluble T cell receptor (sTCR), which method comprises:

25 incubating a host cell which comprises a vector comprising a nucleic acid molecule encoding (i) all or part of a TCR α chain, except the transmembrane domain thereof, and a host cell which comprises a vector comprising a nucleic acid molecule encoding (ii) all or part of a TCR β chain, except the transmembrane domain thereof under conditions causing expression of (i) and (ii), wherein (i) and (ii) each comprise a functional variable domain and
30 at least a part of the constant domain of the TCR chain;

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purifying (i) and (ii); and

mixing (i) and (ii) under refolding conditions such that they are linked by a disulphide bond between constant domain residues which is not present in native TCR.

5

39. A method as claimed in claim 38, wherein one or both of (i) and (ii) comprise all of an extracellular constant Ig domain of the TCR chain.

10

40. A method as claimed in claim 38 or claim 39, wherein one or both of (i) and (ii) comprise all of an extracellular domain of the TCR chain.

41. A method for obtaining a soluble $\alpha\beta$ -form T cell receptor (sTCR), which method comprises:

15

incubating a host cell which comprises a vector comprising a nucleic acid molecule encoding a TCR α chain and a host cell which comprises a vector comprising a nucleic acid molecule encoding a TCR β chain under conditions causing expression of the respective TCR chains;

purifying the respective TCR chains; and

20

mixing the respective TCR chains under refolding conditions such that a covalent disulphide bond links a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain.

25

42. A method as claimed in any one of claims 38 to 41, wherein an interchain disulphide bond in native TCR is not present.

43. A method as claimed in claim 42, wherein native α and β TCR chains are truncated at the C-terminus such that the cysteine residues which form the native interchain disulphide bond are excluded.

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44. A method as claimed in claim 42, wherein cysteine residues which form the native interchain disulphide bond are substituted to another residue.

45. A method as claimed in claim 44, wherein cysteine residues which form the native
5 interchain disulphide bond are substituted to serine or alanine.

46. A method as claimed in any one of claims 38 to 45, wherein an unpaired cysteine residue present in native TCR β chain is not present.

10 47. A method as claimed in any one of claims 38 to 46, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for residues whose β carbon atoms are less than 0.6 nm apart in the native TCR structure.

48. A method as claimed in any one of claims 38 to 47, wherein the disulphide bond
15 which is not present in native TCR is between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01.

49. A method as claimed in any one of claims 38 to 47, wherein the disulphide bond
which is not present in native TCR is between cysteine residues substituted for Thr 45 of exon
20 1 of TRAC*01 and Ser 77 of exon 1 of TRBC1*01 or TRBC2*01.

50. A method as claimed in any one of claims 38 to 47, wherein the disulphide bond
which is not present in native TCR is between cysteine residues substituted for Tyr 10 of exon
1 of TRAC*01 and Ser 17 of exon 1 of TRBC1*01 or TRBC2*01.

25

51. A method as claimed in any one of claims 38 to 47, wherein the disulphide bond
which is not present in native TCR is between cysteine residues substituted for Thr 45 of exon
1 of TRAC*01 and Asp 59 of exon 1 of TRBC1*01 or TRBC2*01.

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52. A method as claimed in any one of claims 38 to 47, wherein the disulphide bond which is not present in native TCR is between cysteine residues substituted for Ser 15 of exon 1 of TRAC*01 and Glu 15 of exon 1 of TRBC1*01 or TRBC2*01.

5 53. A method as claimed in any one of claims 38, 39 and 42 to 52, wherein (i) and (ii) each comprise the functional variable domain of a first TCR fused to all or part of the constant domain of a second TCR, the first and second TCRs being from the same species.

10 54. A method as claimed in claim 53, wherein the constant domains of the second TCR are truncated N-terminal to the residues which form the non-native interchain disulphide bond.

55. A method as claimed in any one of claims 38 to 54, wherein one or both of the chains are derivatised with, or fused to, a moiety at its C or N terminus.

15

56. A method as claimed in any one of claims 38 to 55, wherein one or both of the chains have a cysteine residue at its C terminus, its N terminus or both termini, to which a moiety can be fused.

20 57. A method as claimed in any one of claims 38 to 56, wherein the sTCR further comprises a detectable label.

58. A method as claimed in any one of claims 38 to 57, wherein the sTCR is associated with a therapeutic agent.

25

59. A method as claimed in any one of claims 36 to 58, further comprising combining a plurality of sTCRs to form a multivalent T cell receptor (TCR) complex.

30 60. A method as claimed in claim 59, wherein the sTCRs are combined to form a sTCR multimer.

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61. A method as claimed in claim 60, wherein two or three or four or more T cell receptor molecules are associated with one another.

62. The method of claim 61, wherein two or more of said T cell receptor molecules are
5 associated via a linker molecule.

63. A method as claimed in any one of claims 59 to 62, wherein the sTCRs or sTCR multimers are combined in a lipid bilayer or are attached to a particle.

10 64. A method for detecting MHC-peptide complexes, which comprises:

(i) providing a soluble TCR produced by the method of any one of claims 38 to 58 or a multivalent T cell receptor complex produced by the method of any one of claims 59 to 63;

(ii) contacting the soluble TCR or multivalent TCR complex with the MHC-peptide complexes; and
15

(iii) detecting binding of the soluble TCR or multivalent TCR complex to the MHC-peptide complexes.

65. A pharmaceutical formulation comprising a sTCR produced by the method of any one
20 of claims 36 to 58, a multivalent TCR complex produced by the method of any one of claims 59 to 63, or both of said sTCR and multivalent TCR complex, together with a pharmaceutically acceptable carrier.

Figure 1

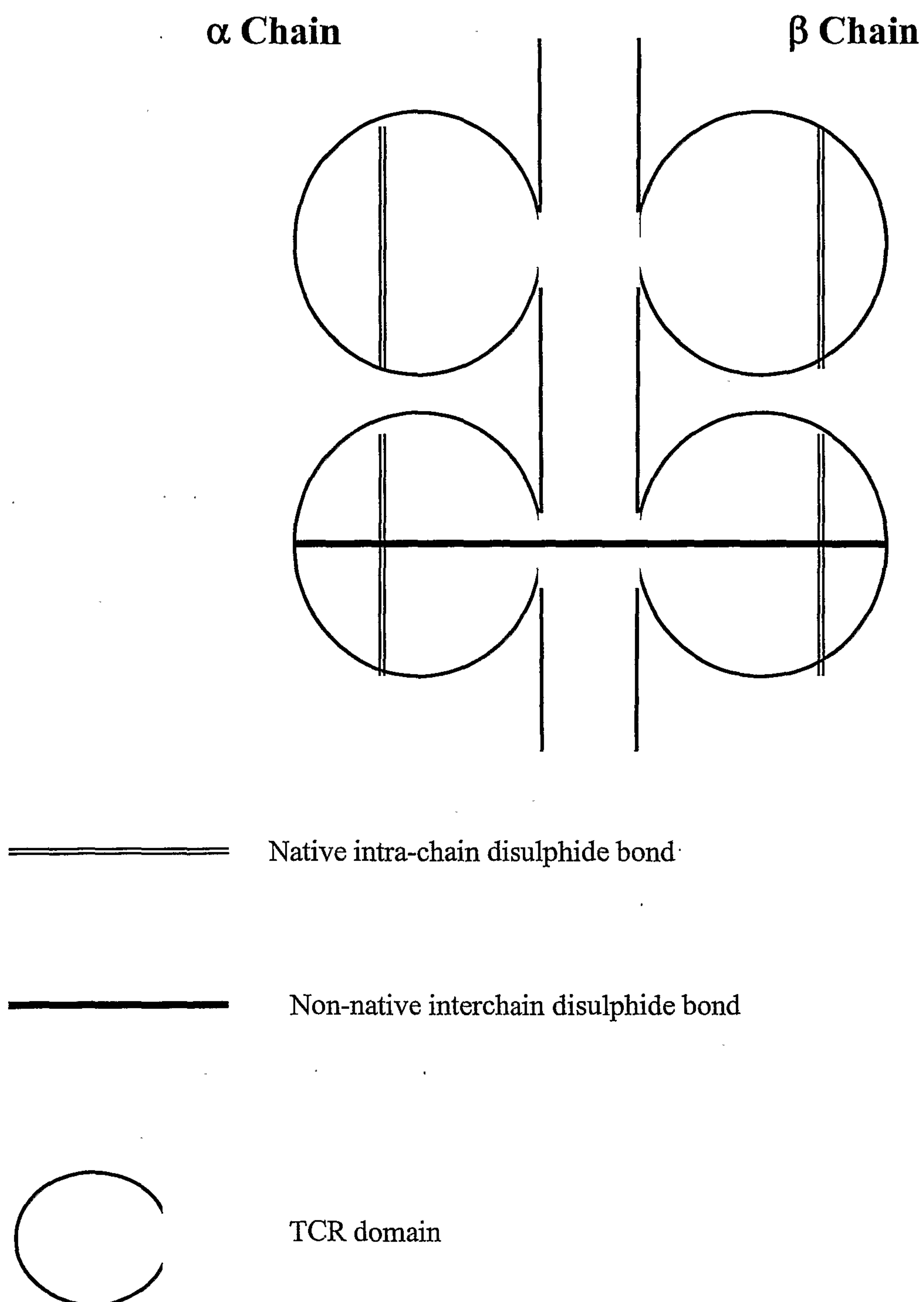


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Figure 2b

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Figure 3a

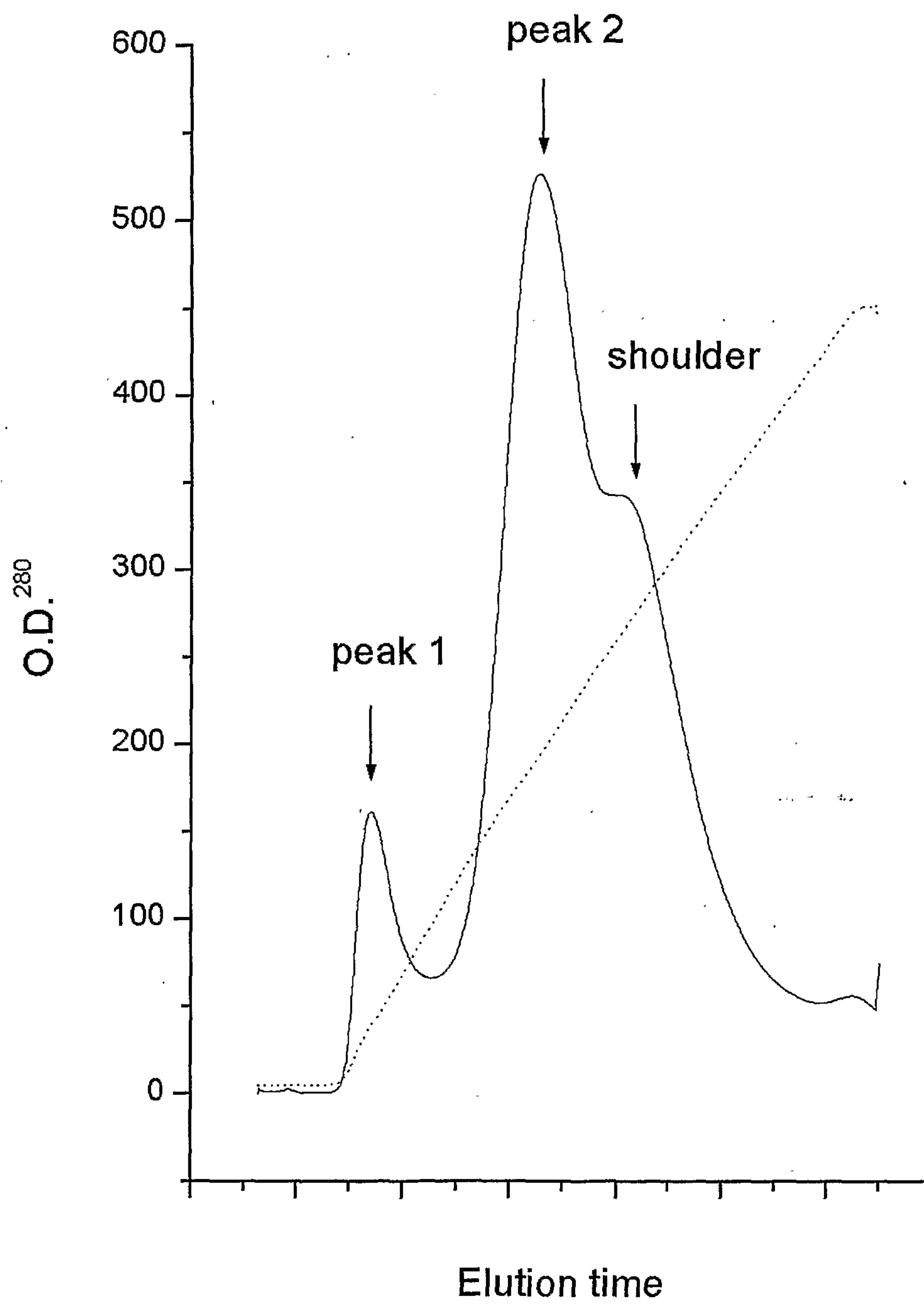
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Figure 3b

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Figure 4



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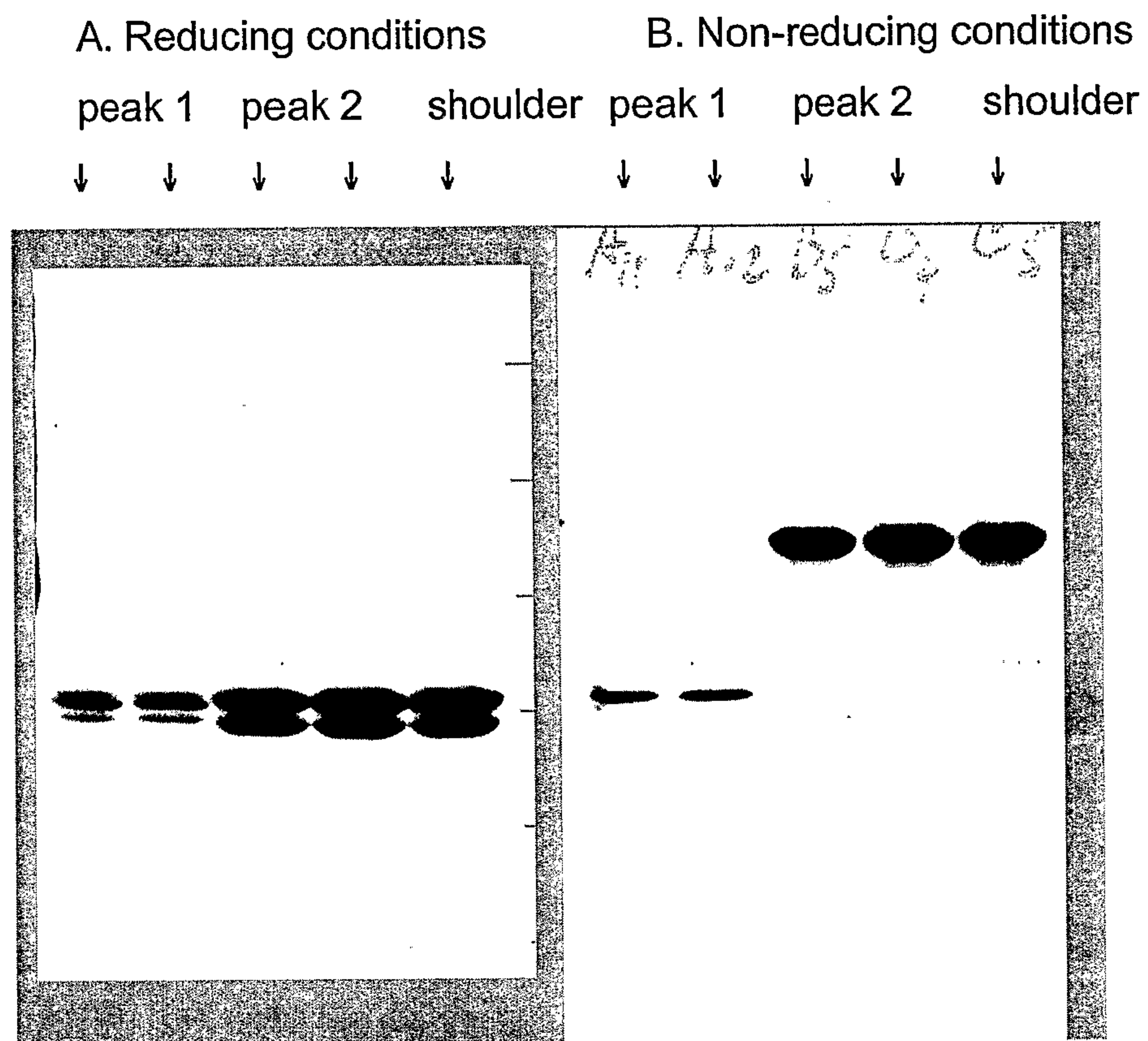
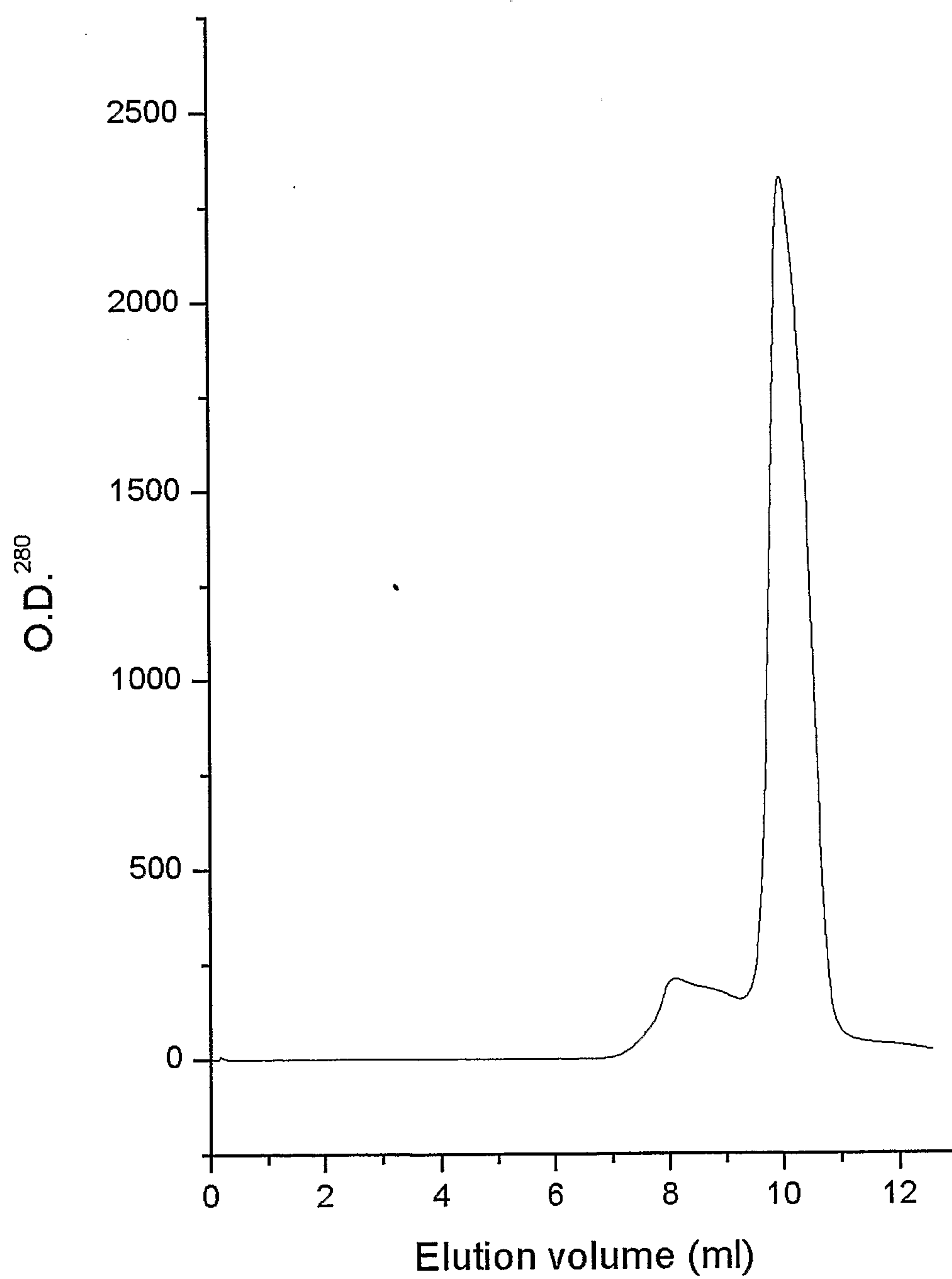


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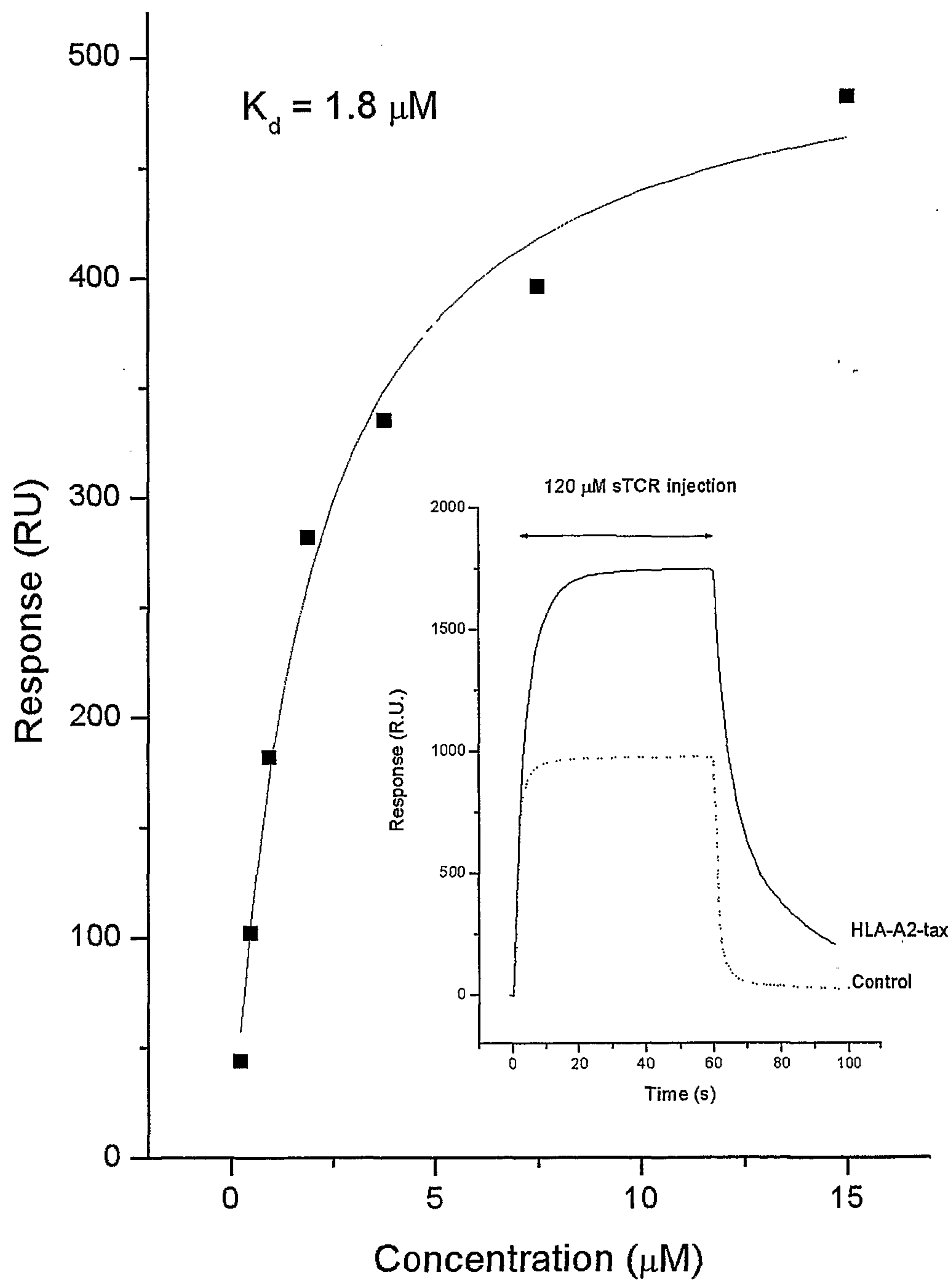
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Figure 6



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Figure 7



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Figure 8a

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tatcacagacaaatgtgtgctagacatgaggtctatggacttcaagagcaacagtgct
gtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaacaacagcatta
ttccagaagacaccttcttccccagcccagaaagttcctaa

Figure 8b

atgcaactactagaacaaagtcctcagtttctaagcatccaagagggagaaaaatctca
ctgtgtactgcaactcctcaagtgttttttccagcttacaatggtacagacaggagcc
tggggaagggtcctgtcctcctggtgacagtagttacgggtggagaagtgaagaagctg
aagagactaacctttcagtttgggtgatgcaagaaaggacagttctctccacatcactg
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cctgccgtgtaccagctgagagactctaaatccagtgacaagtctgtctgcctattca
ccgatttttgattctcaaacaatgtgtcaciaaagtaaggattctgatgtgtatatcac
agacaaatgtgtgctagacatgaggtctatggacttcaagagcaacagtgctgtggcc
tggagcaacaaatctgactttgcatgtgcaaacgccttcaacaacagcattattccag
aagacaccttcttccccagcccagaaagttcctaa

Figure 8c

atggtggatggtggaatcactcagtccccaaagtacctgttcagaaaggaaggacaga
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Figure 9a

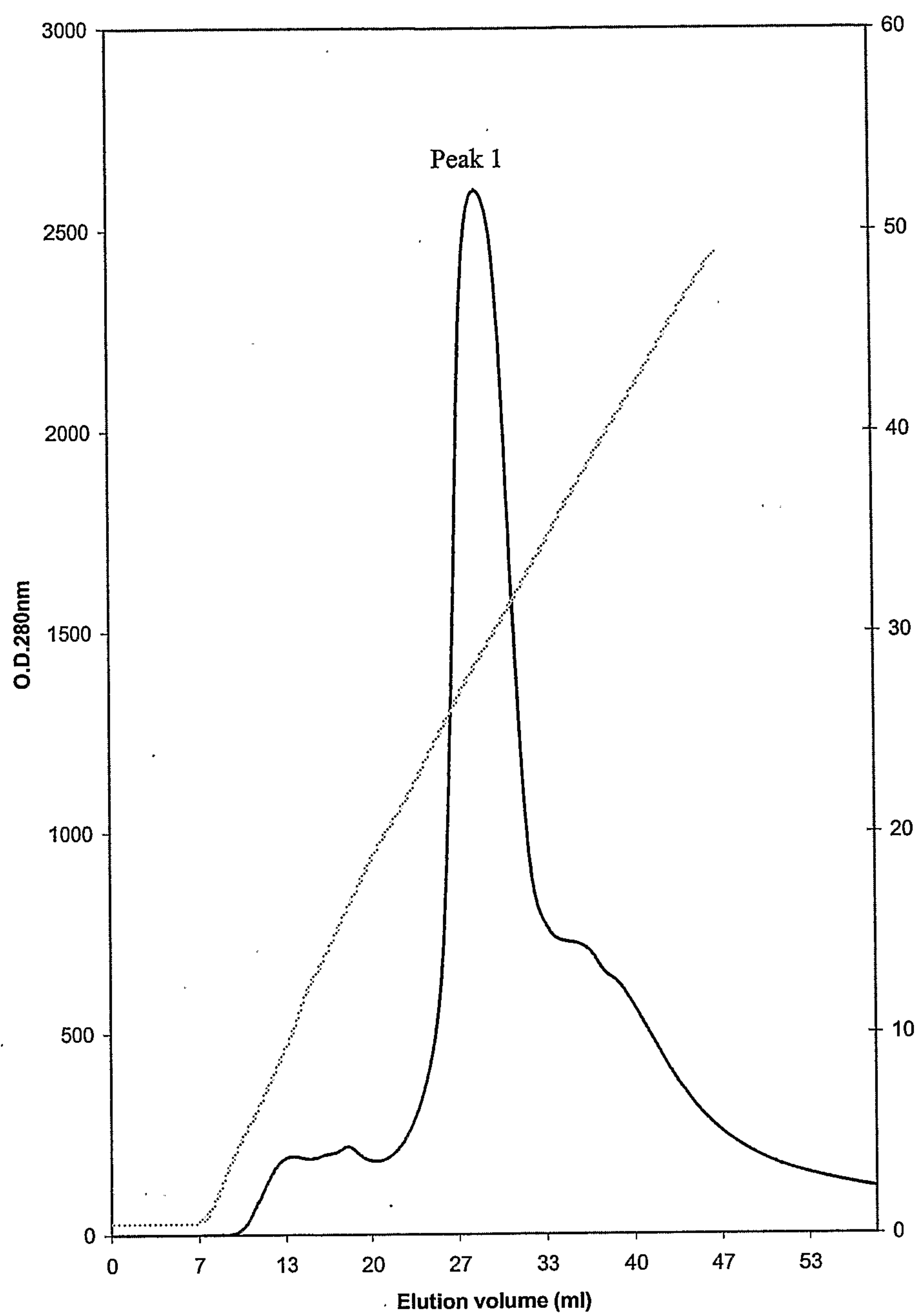
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AAQPGDTGLYLCAGAGSQGNLIFGKGTKLSVKPNIQNP
DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVY
ITDKCVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSI
IPEDTFFPSPESS **Stop**

Figure 9b

MVDGGITQSPKYLFRKEGQNVTLSC EQNLNHDAMYWY
RQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESF
PLTVTSAQKNPTAFYLCASSSRSSYEQYFGPGTRLTVTE
DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDH
VELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYSLSS
RLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRAD **Stop**

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Figure 10



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Figure 11

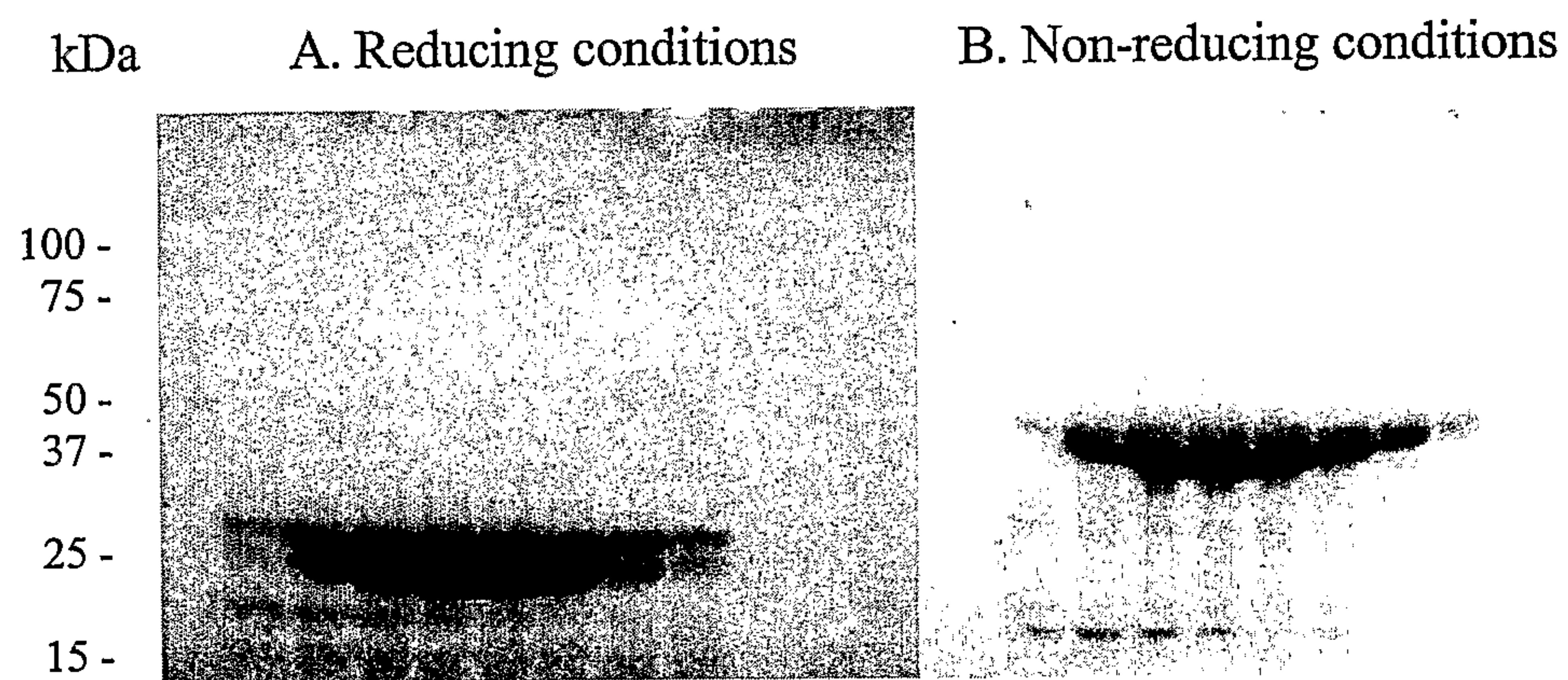
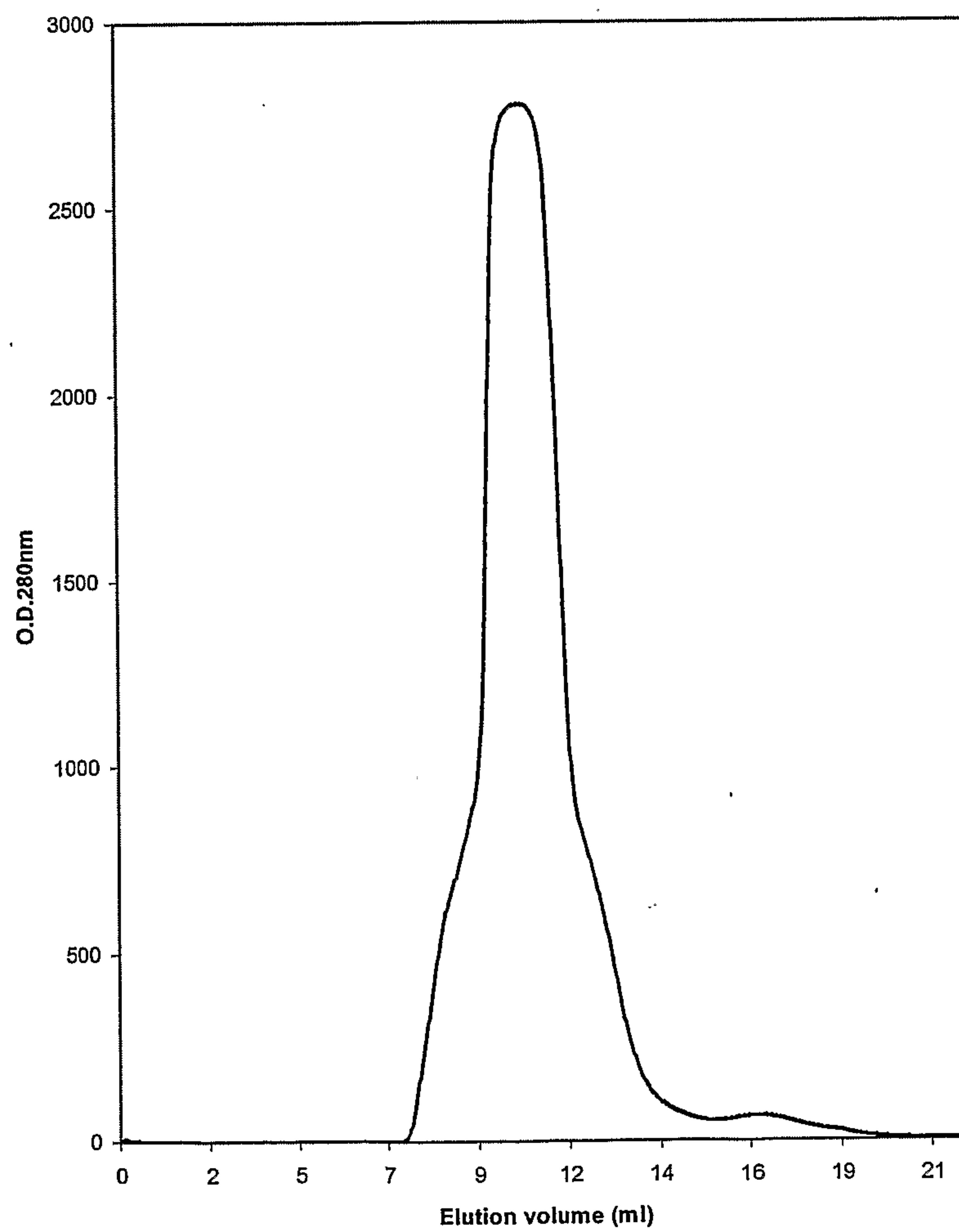


Figure 12



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Figure 13a

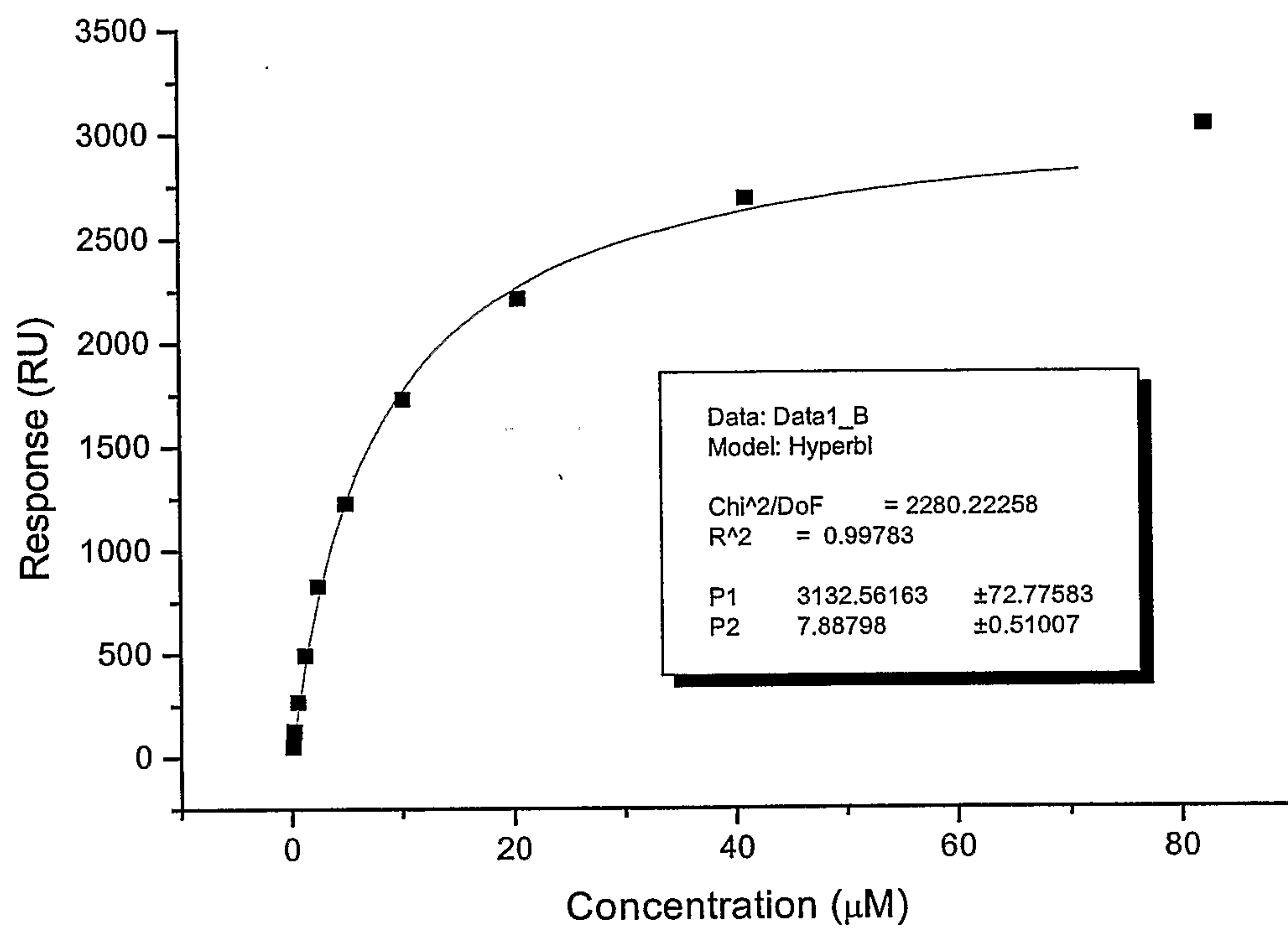
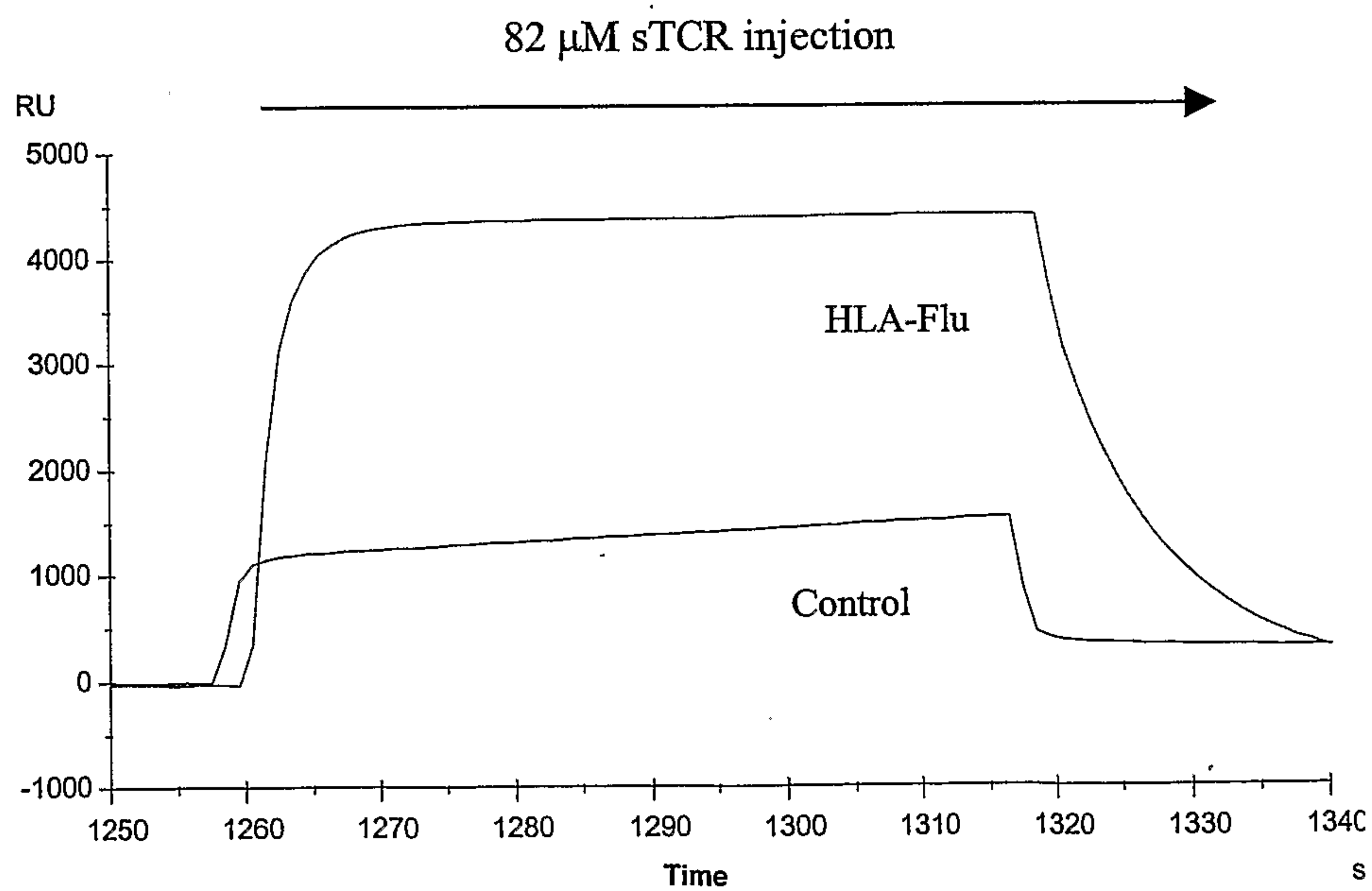


Figure 13b



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Figure 14a

atgcaggaggygacacagattcctgcagctctgagtgtcccagaaggagaaaacttgg
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 ccctgggaaaggtctcacatctctgttgcttattcagtcagtcagagagagcaaaca
 agtggaagacttaatgcctcgctggataaatcatcaggacgtagtactttatacattg
 cagcttctcagcctgggtgactcagccacctacctctgtgctgtgaggcccacatcagg
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 cagaaccctgaccctgccgtgtaccagctgagagactctaaatccagtgacaagtctg
 tctgcctattcaccgattttgattctcaaacaatgtgtcacaaagtaaggattctga
 tgtgtatatcacagacaaatgtgtgctagacatgaggtctatggacttcaagagcaac
 agtgctgtggcctggagcaacaatctgactttgcatgtgcaaacgccttcaacaaca
 gcattattccagaagacaccttcttccccagcccagaaagttcctaa

Figure 14b

atgggtgtcactcagacccccaaaattccaggtcctgaagacaggacagagcatgacac
 tgcagtgtgcccaggatatgaaccatgaatacatgtcctgggtatcgacaagaccagg
 catggggctgaggctgattcattactcagttgggtgctgggtatcactgaccaaggagaa
 gtccccaatggctacaatgtctccagatcaaccacagaggatttcccgtcaggctgc
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 caccggggagctgttttttggagaaggctctaggctgaccgtactggaggacctgaaa
 aacgtgttcccacccgaggtcgctgtgtttgagccatcagaagcagagatctcccaca
 cccaaaaggccacactgggtgtgcctggccacaggcttctaccccgaccacgtggagct
 gagctgggtgggtgaatgggaaggaggtgcacagtgggggtctgcacagacccgcagccc
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 tctcggccaccttctggcaggacccccgcaaccacttccgctgtcaagtccagttcta
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Figure 15a

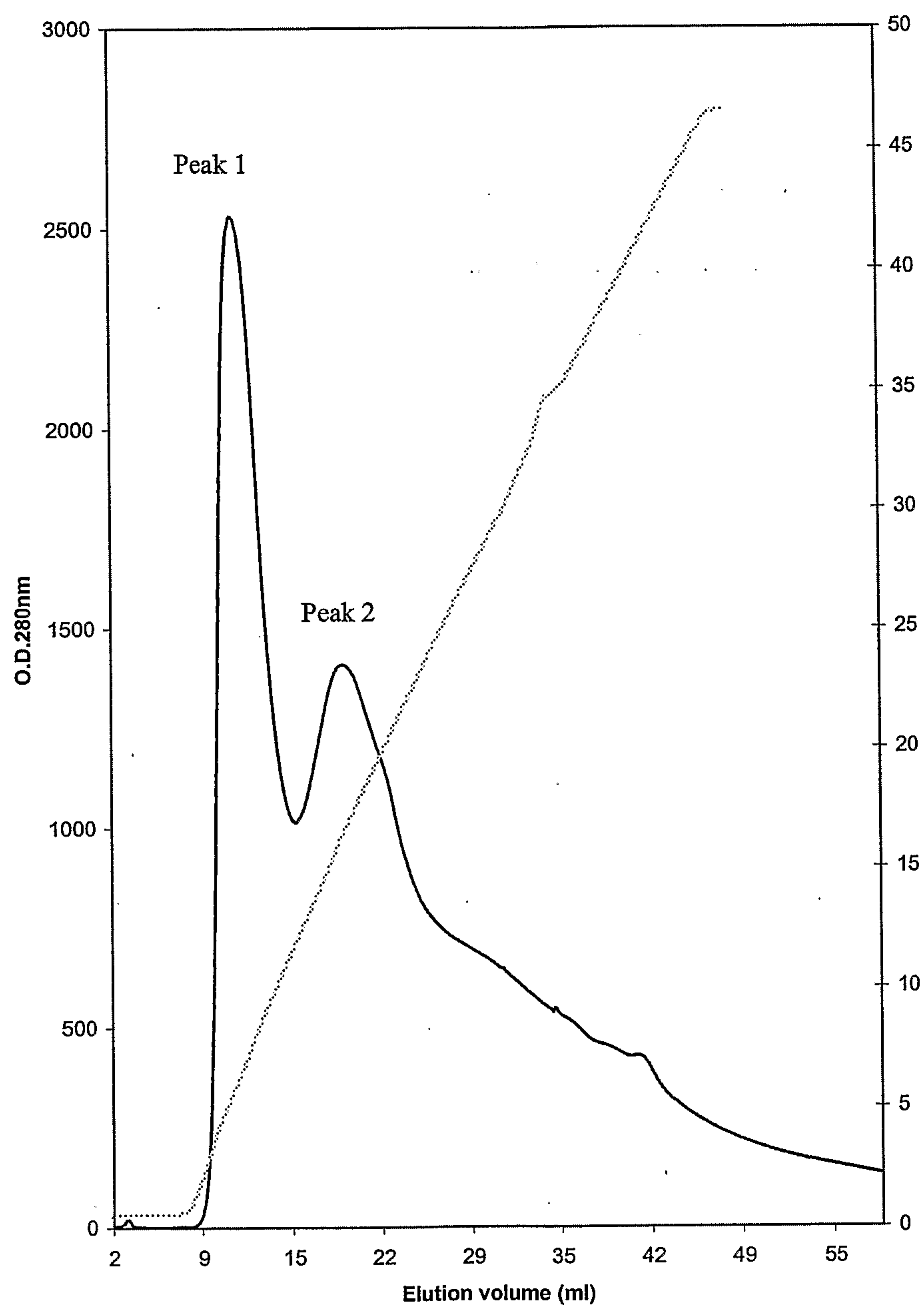
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 AASQPGDSATYLC AVRPTSGGSYIPTFGRGTS LIVHPYI
 QNPDP AVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDS
 DVIYITDKCVLDMRSMDFKSNSA VAWSNKSDFA CANAF
 NNSIIPEDTFFPSP ESS Stop

Figure 15b

MGV TQTPKFQVLKTGQSM TLQCAQDMNHEYMSWYRQ
 DPG Met GLRLIHYSVGAGITDQGEVPNGYNVSRSTTEDF
 PLRLLSAAP SQTSVYFCASSYVGNTGELFFGEGSRLTVL
 EDLKNVFPPEVA VFEPSEAEISHTQKATLVCLATGFYPD
 HVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYAL
 SSR LRVSATFWQDPRNHFR CQVQFYGLSENDEWTQDRA
 KPVTQIVSAEAWGRAD Stop

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Figure 16



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Figure 17

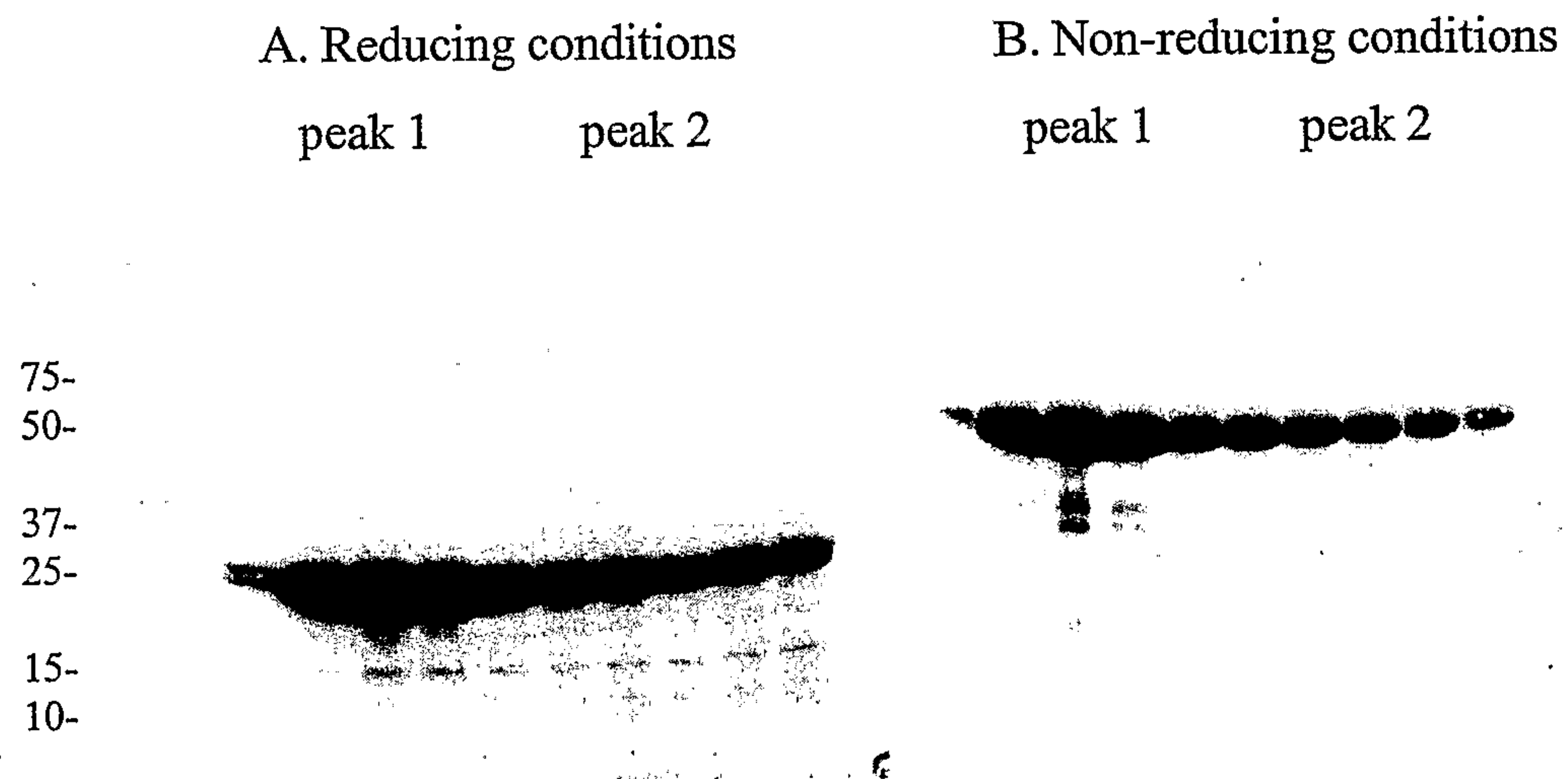
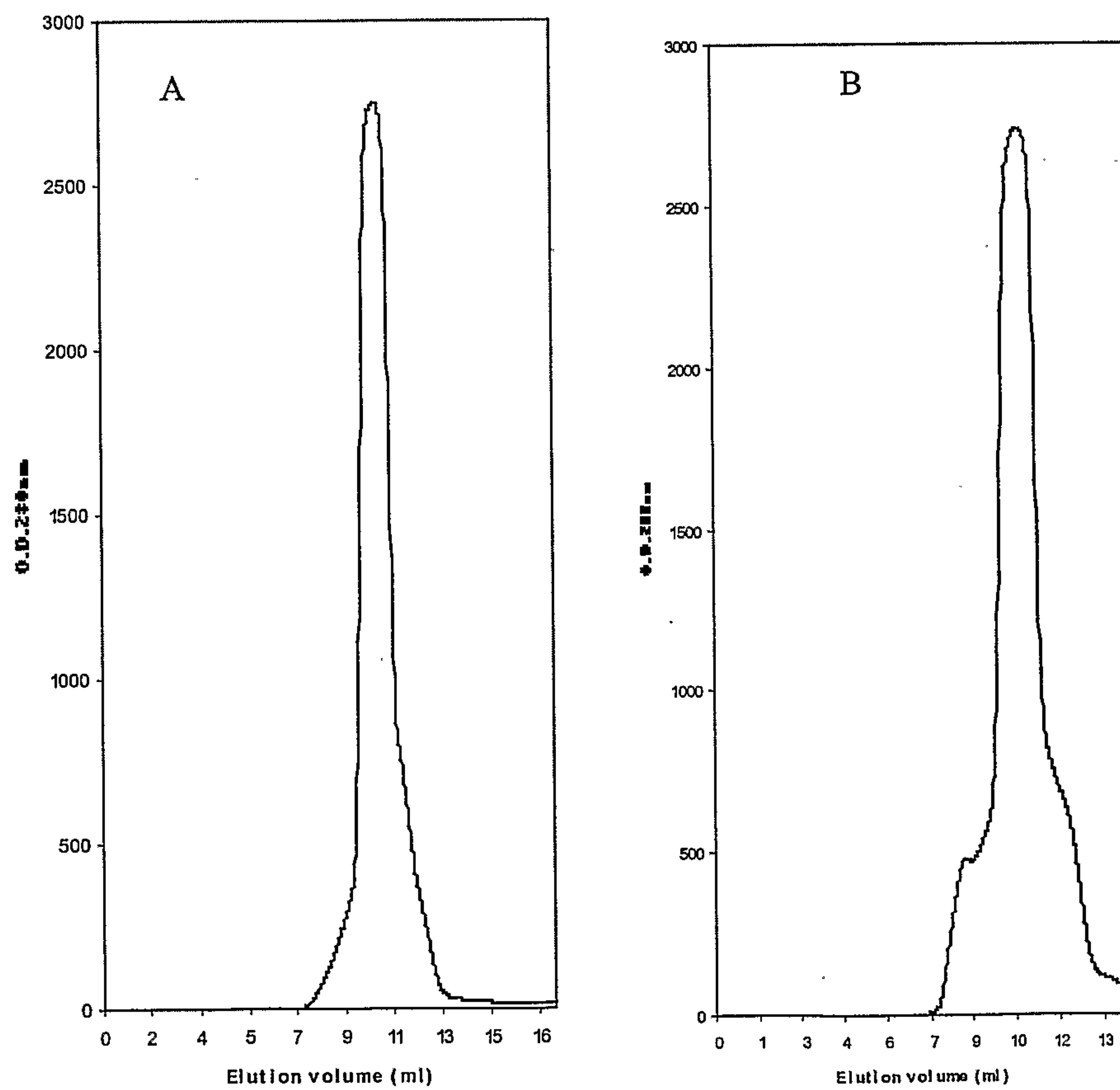


Figure 18



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Figure 19a

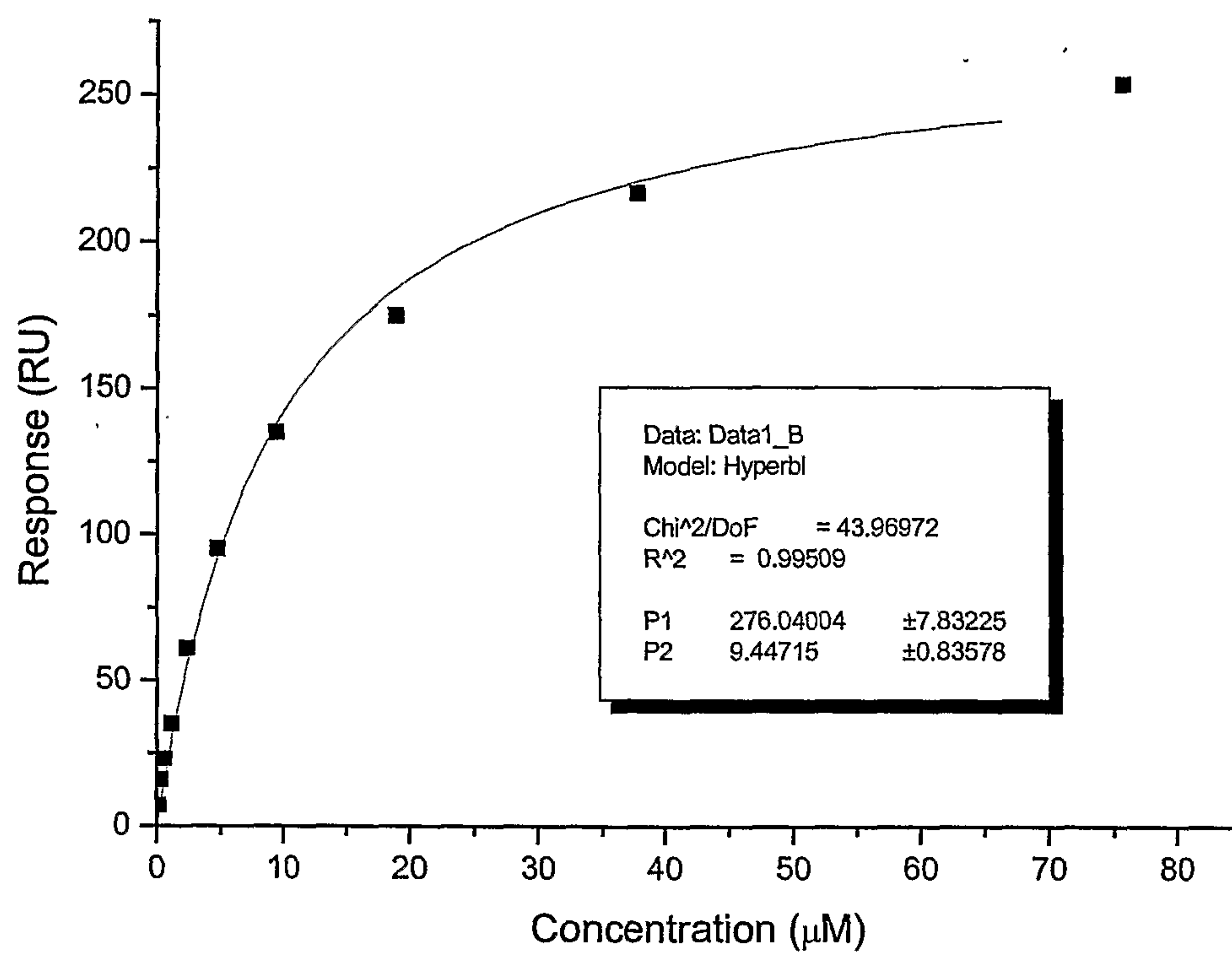
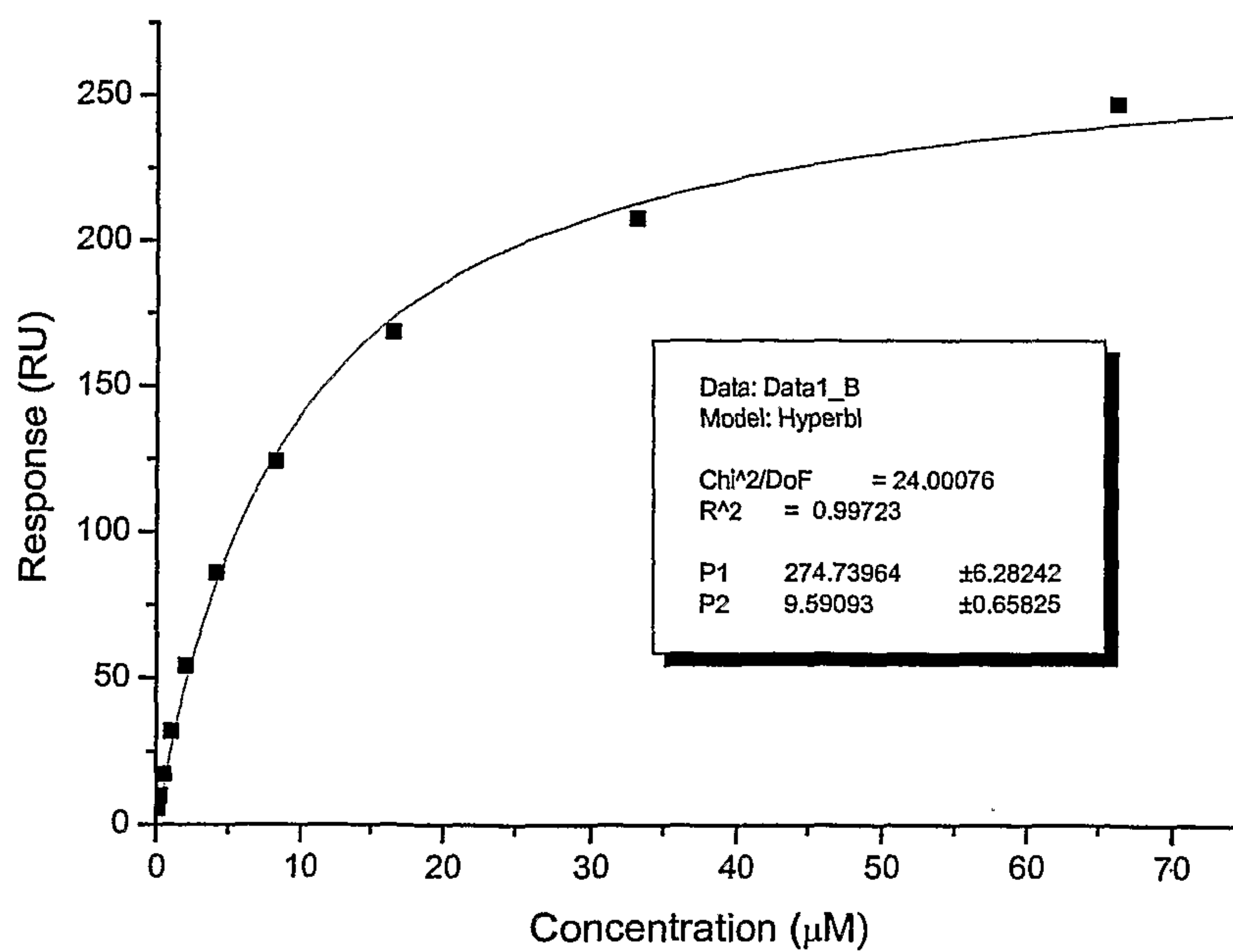


Figure 19b



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Figure 20a

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ccctgggaaagggtctcacatctctgttgcttattcagtcaagtcagagagagcaaaca
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cagcttctcagcctgggtgactcagccacctacctctgtgctgtgaggcccacatcagg
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cagaaccttgacctgccgtgtaccagctgagagactctaaatccagtgacaagtctg
tctgcctattcaccgatttttgattctcaacaaatgtgtcacaaagtaaggattctga
tgtgtatatcacagacaaaaggtgtgctagacatgaggtctatggacttcaagagcaac
agtgtgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaacaaca
gcattattccagaagacaccttcttccccagcccagaaagttcctgttaa

Figure 20b

atgggtgtcactcagacccccaaaattccagggtcctgaagacaggacagagcatgacac
tgcagtgtgcccaggatatgaacctgaatacatgtcctgggtatcgacaagacctagg
catggggctgaggctgattcattactcagttgggtgctgggtatcactgaccaaggagaa
gtccccaatgggtacaatgtctccagatcaaccacagaggatttcccgtcaggctgc
tgtcgggtgctccctcccagacatctgtgtacttctgtgccagcagttacgtcgggaa
caccggggagctgttttttggagaaggctctaggctgacctactggaggacctgaaa
aacgtgttcccacccgagggtcgctgtgtttgagccatcagaagcagagatctcccaca
cccaaaaggccacactgggtgtgcctggccacaggcttctaccccgaccacgtggagct
gagctgggtgggtgaatgggaaggaggtgcacagtgggggtcagcagacccgcagccc
ctcaaggagcagcccgcctcaatgactccagatacgctctgagcagccgcctgaggg
tctcggccaccttctggcaggacccccgcaaccacttccgctgtcaagtccagttcta
cgggctctcggagaatgacgagtggacccaggatagggccaaaccggtcacccagatc
gtcagcgccgaggcctggggtagagcagactgttaa

Figure 21a

MQEXTQIPAALSVPEGENLVLNCSFTDS AIYNLQWFRQ
DPGKGLTSLLLIQSSQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLC AVRPTSGGSYIPTFGRGTSLIVHPYI
QNPDP AVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDS
DVYITDKCVLDMRSMDFKSNSA VAWSNKSDFACANAF
NNSIIPEDTFFPSP ESSC Stop

Figure 21b

MGVTQTPKFQVLKTGQSM TLQCAQDMNHEYMSWYRQ
DPGMGLRLIHYSVGAGITDQGEVPNGYNVSRSTTEDFP
LRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVLE
DLKNVFPPEVA VFEPSEAEISHTQKATLVCLATGFYPDH
VELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYALSS
RLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAK
PVTQIVSAEAWGRADC Stop

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Figure 22

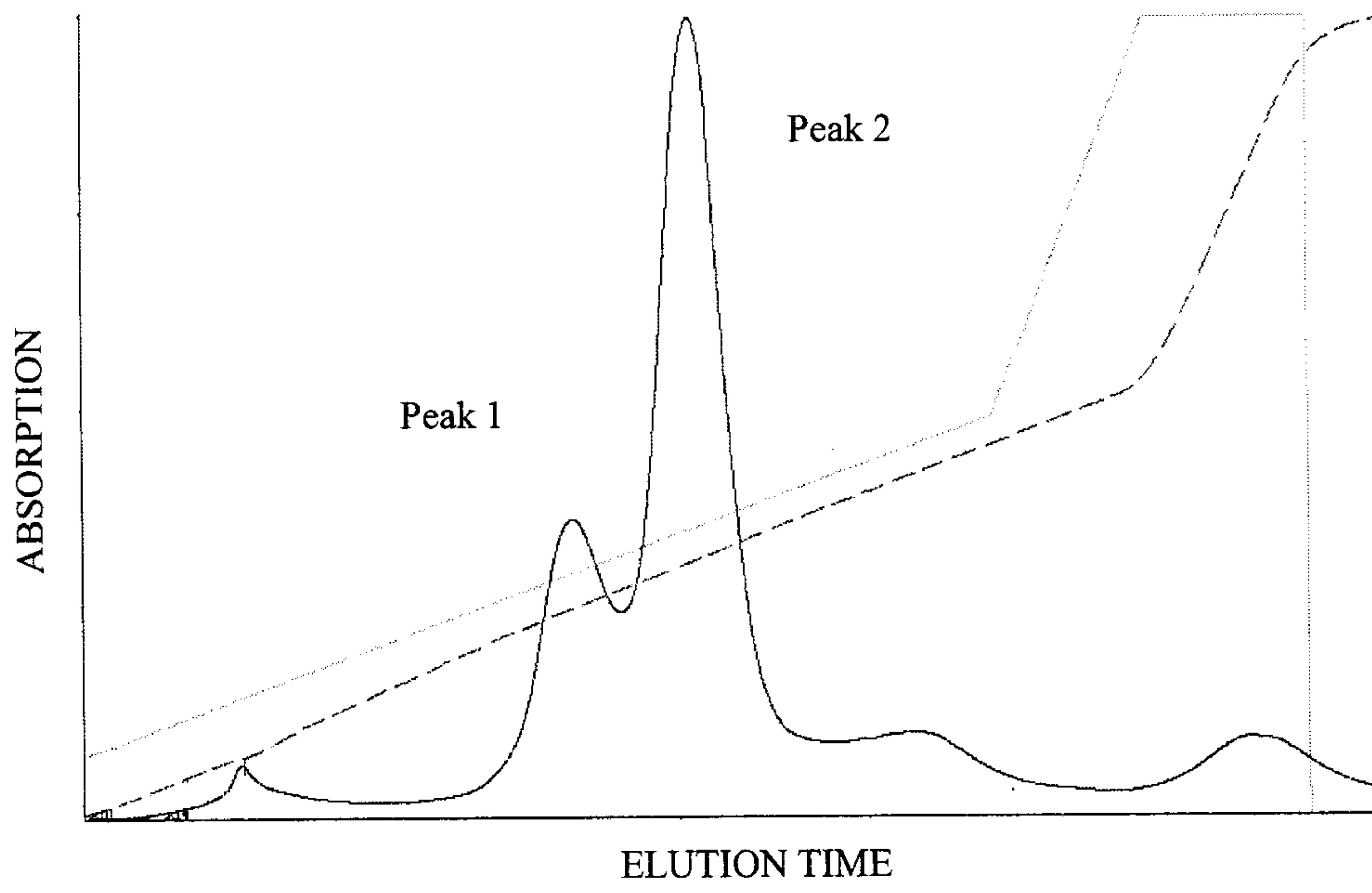
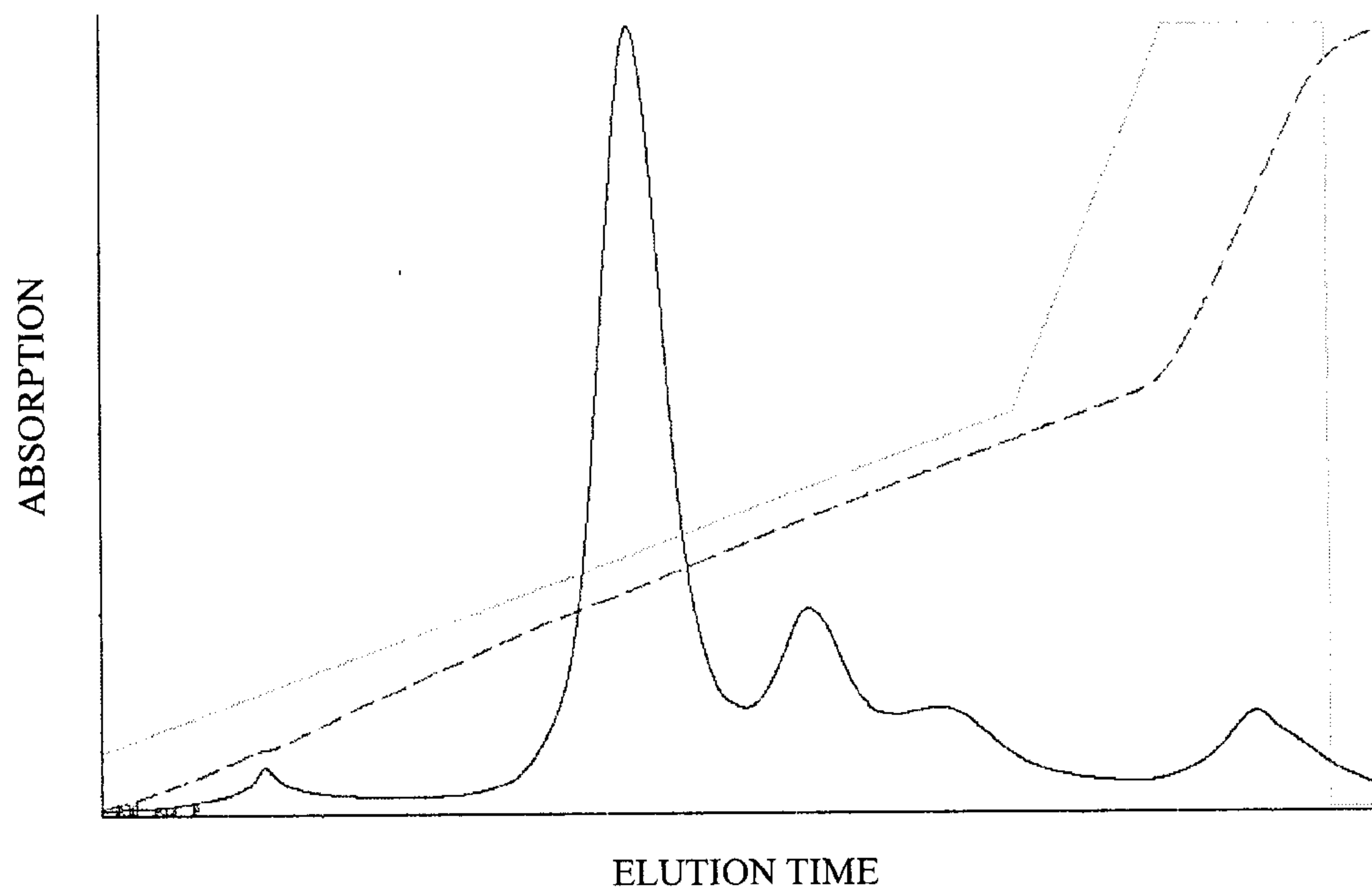
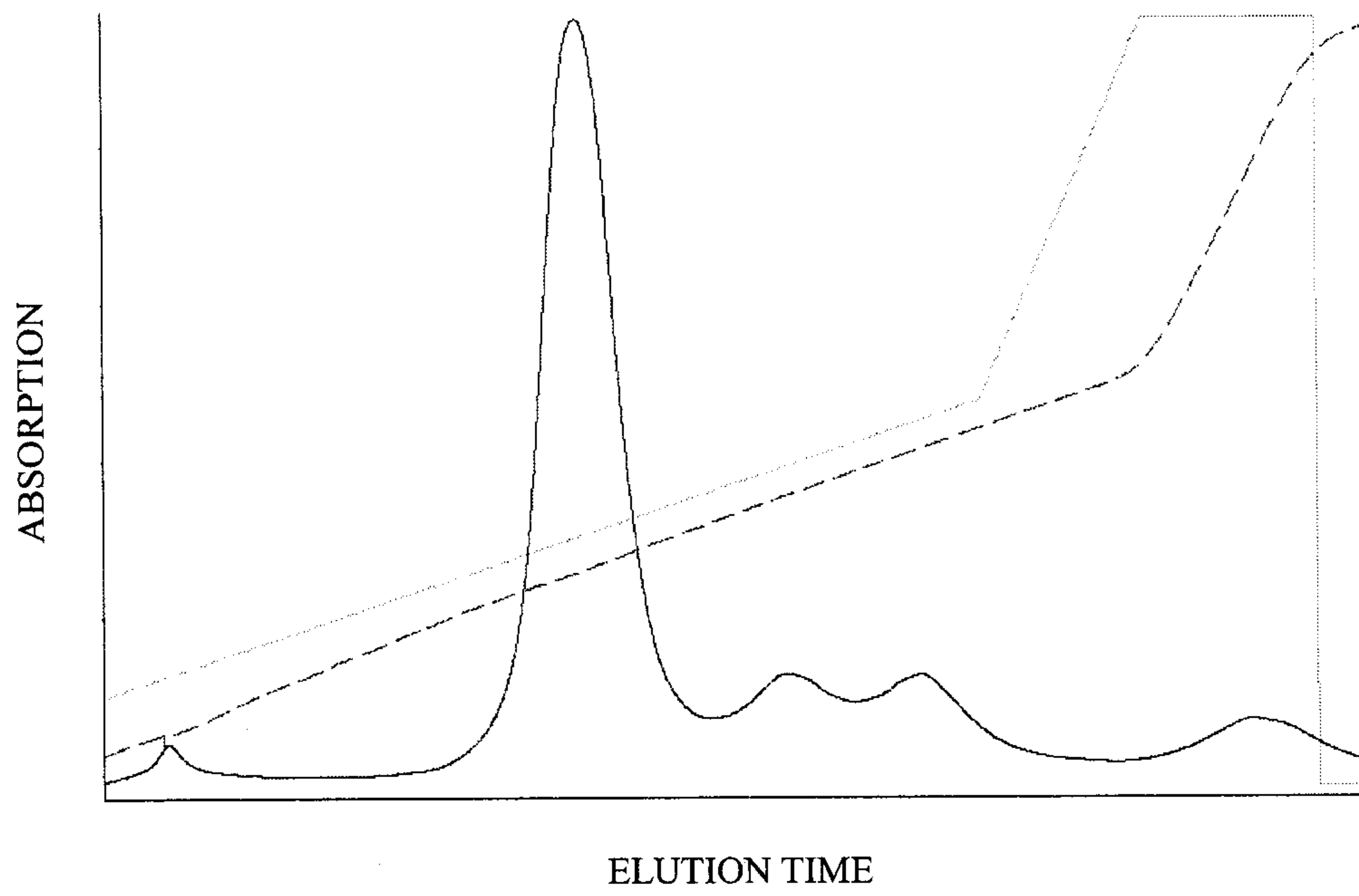


Figure 23



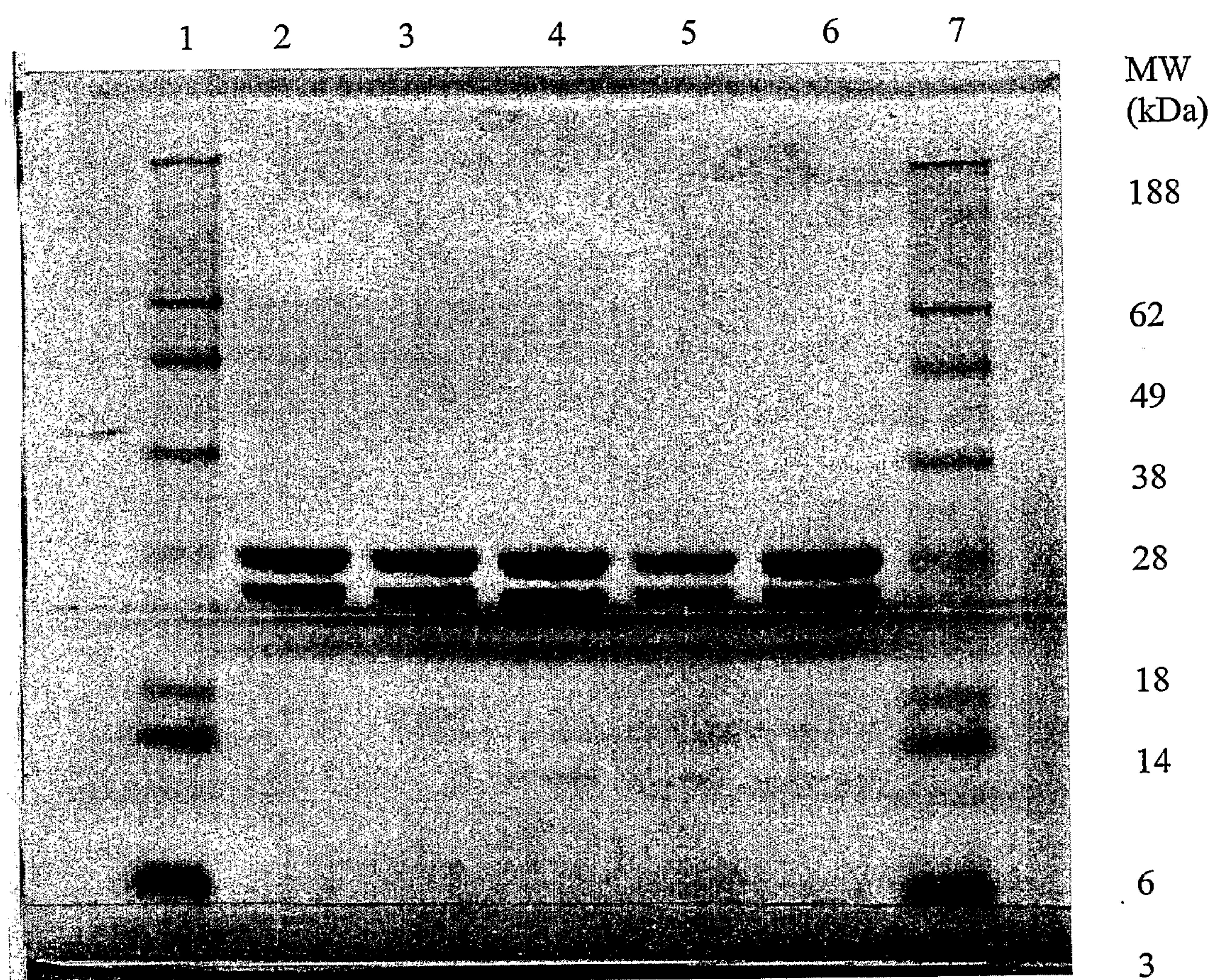
19/81

Figure 24



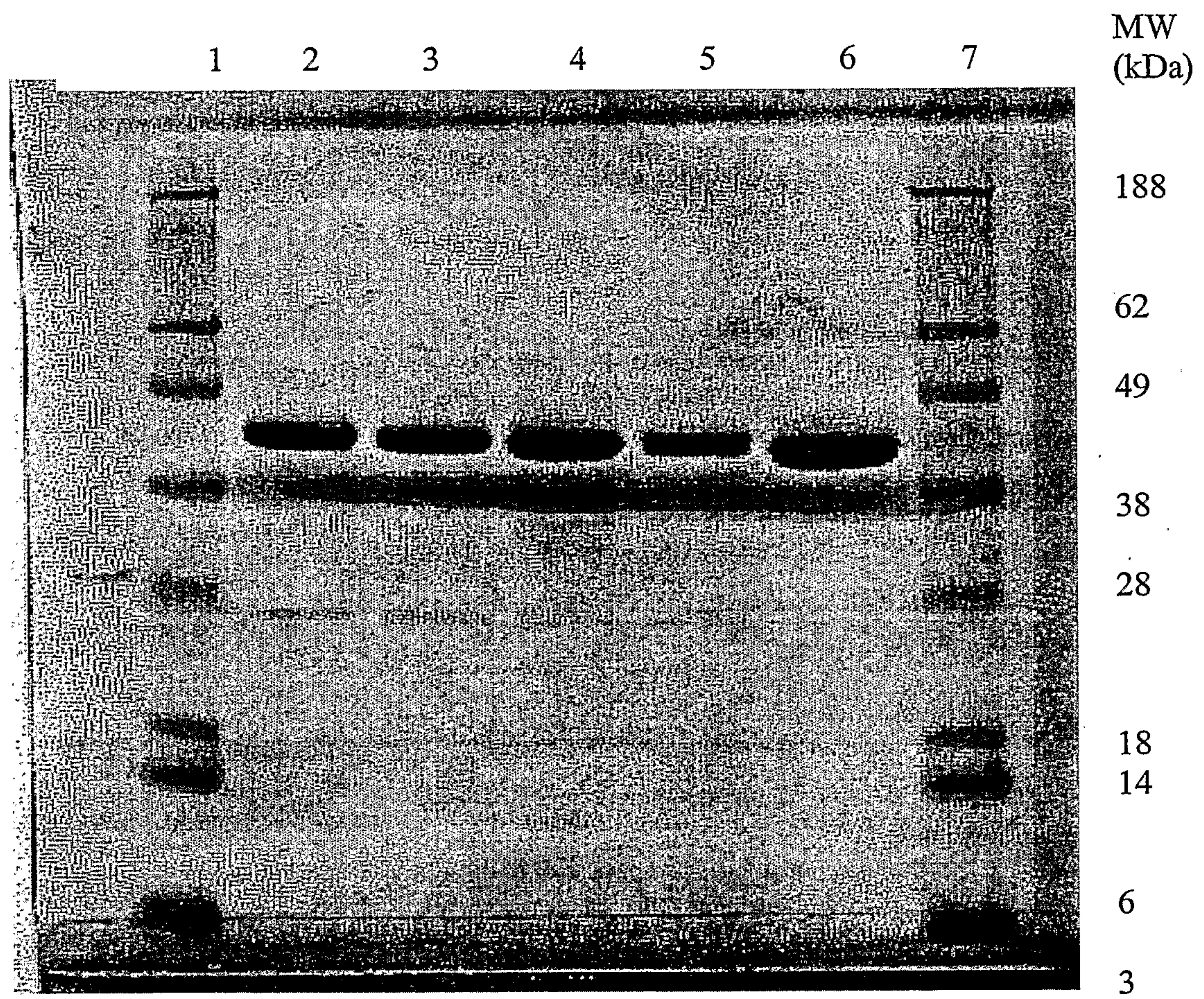
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Figure 25



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Figure 26



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Figure 27

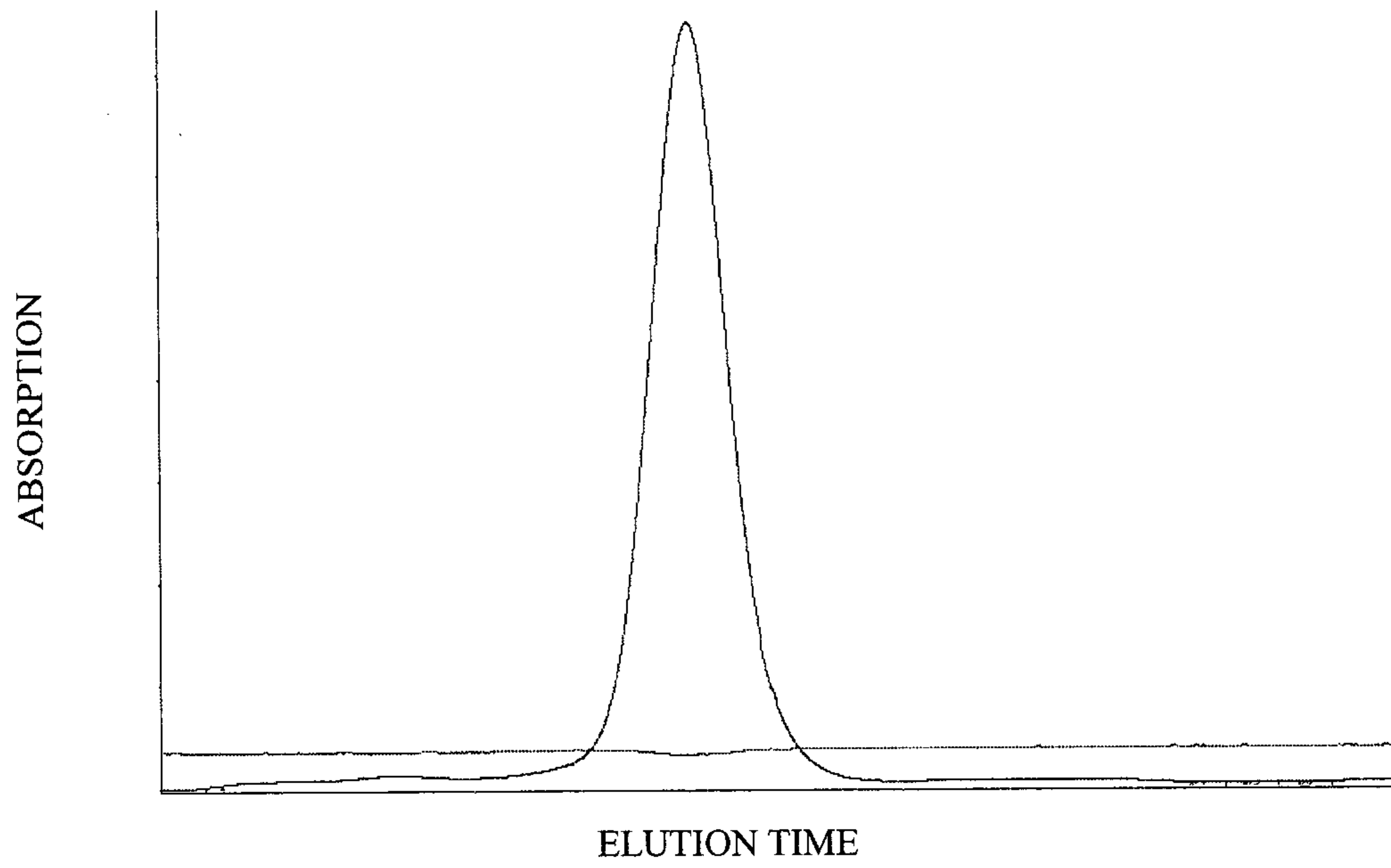
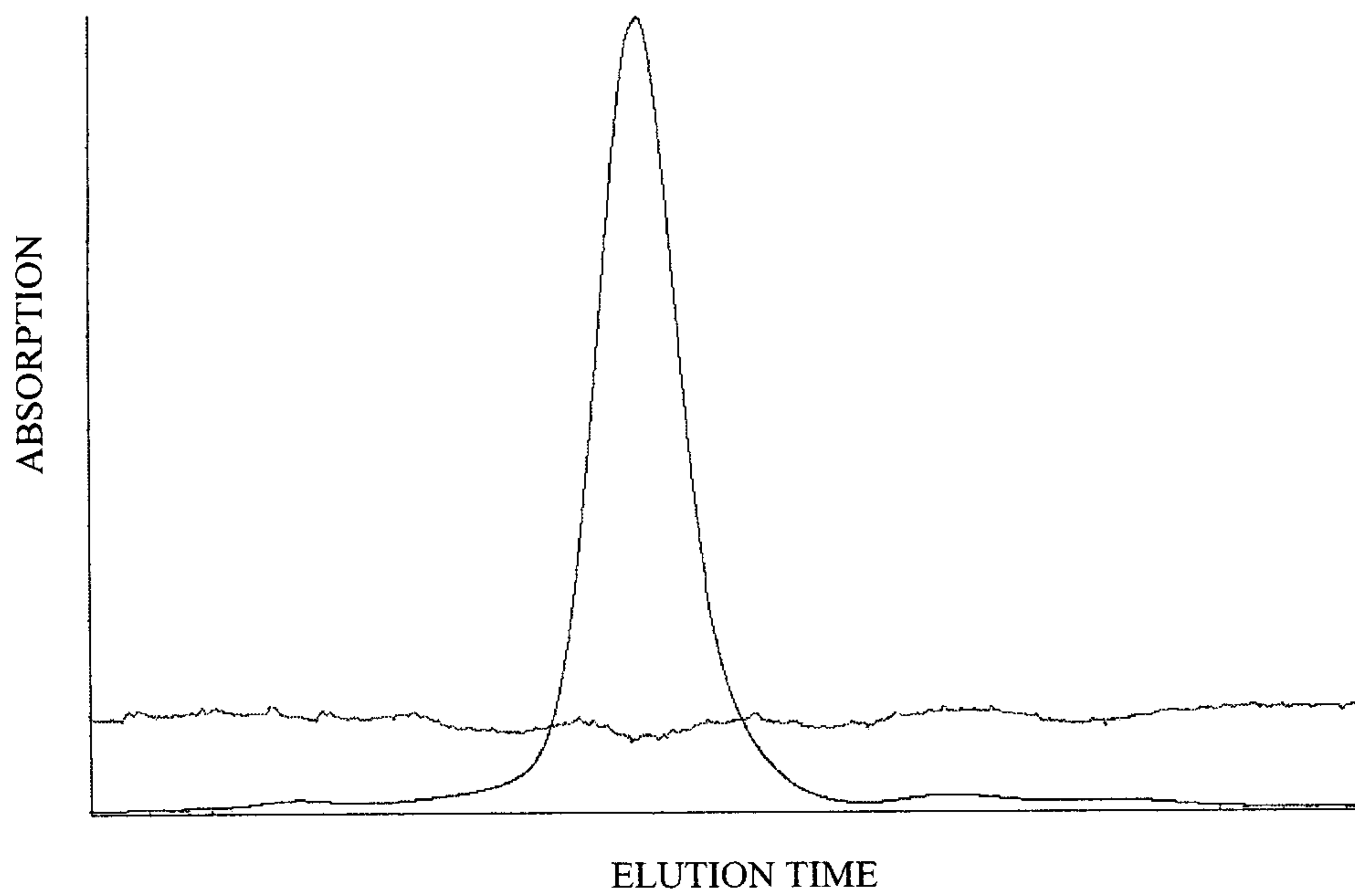
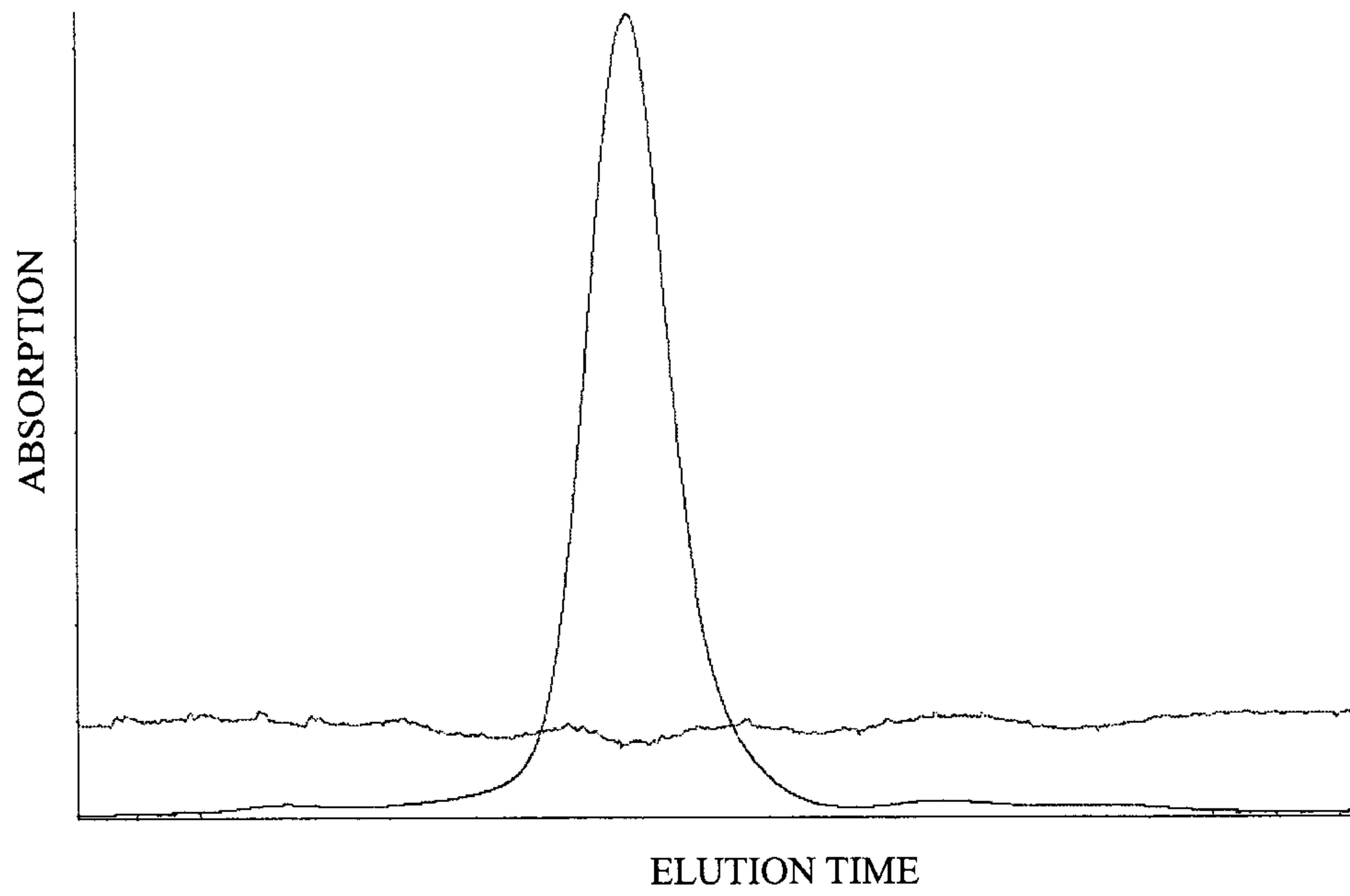


Figure 28



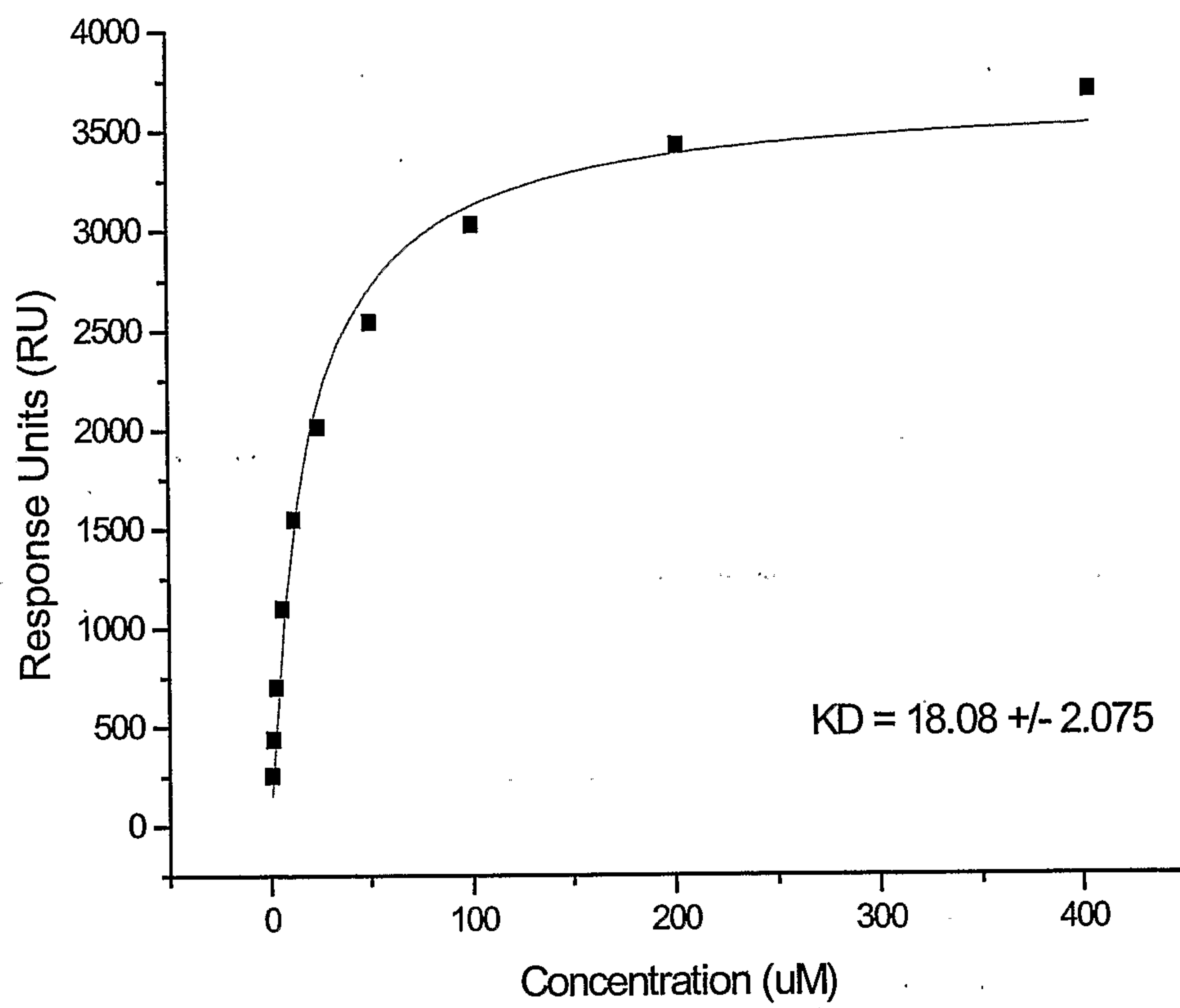
23/81

Figure 29



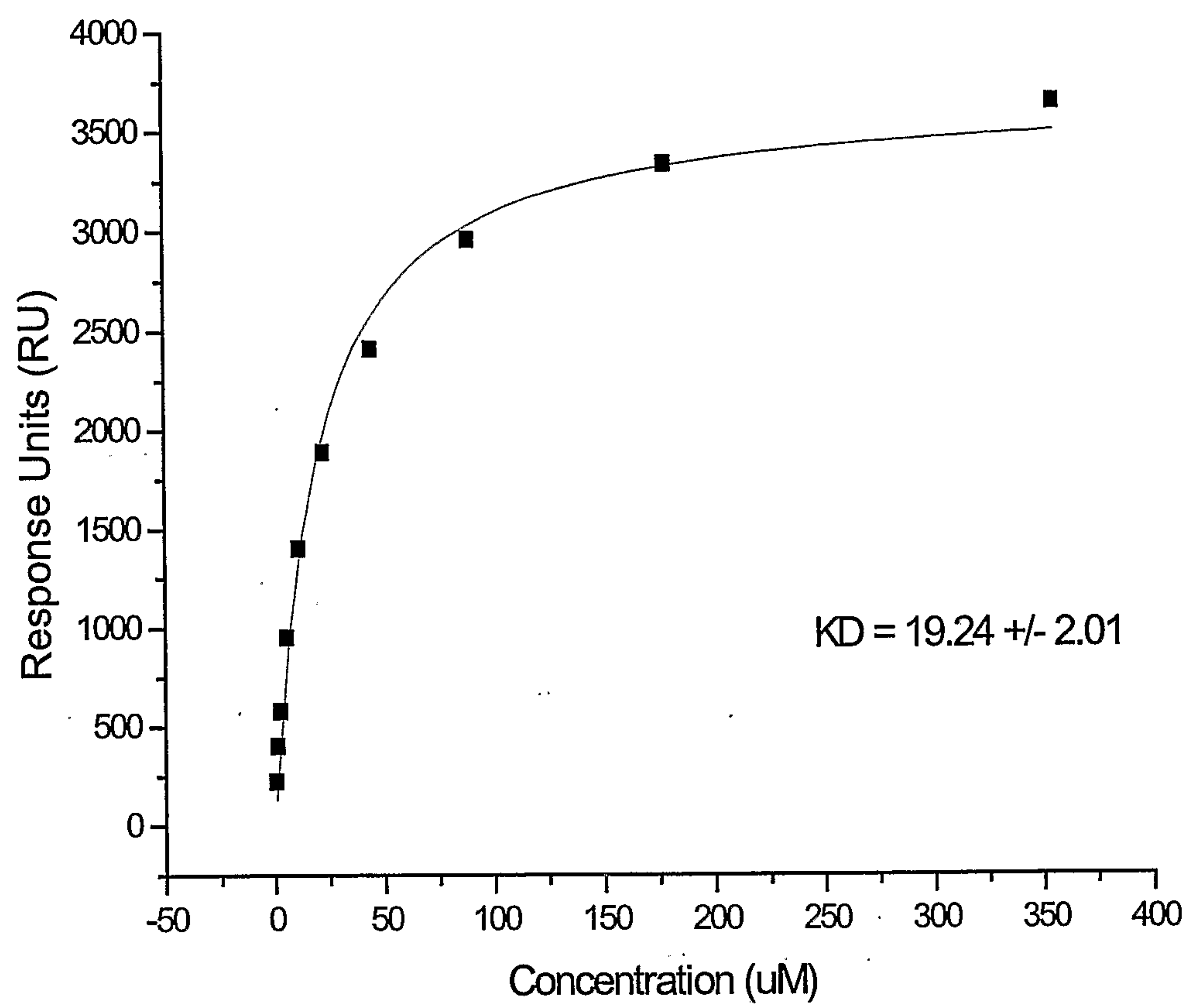
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Figure 30



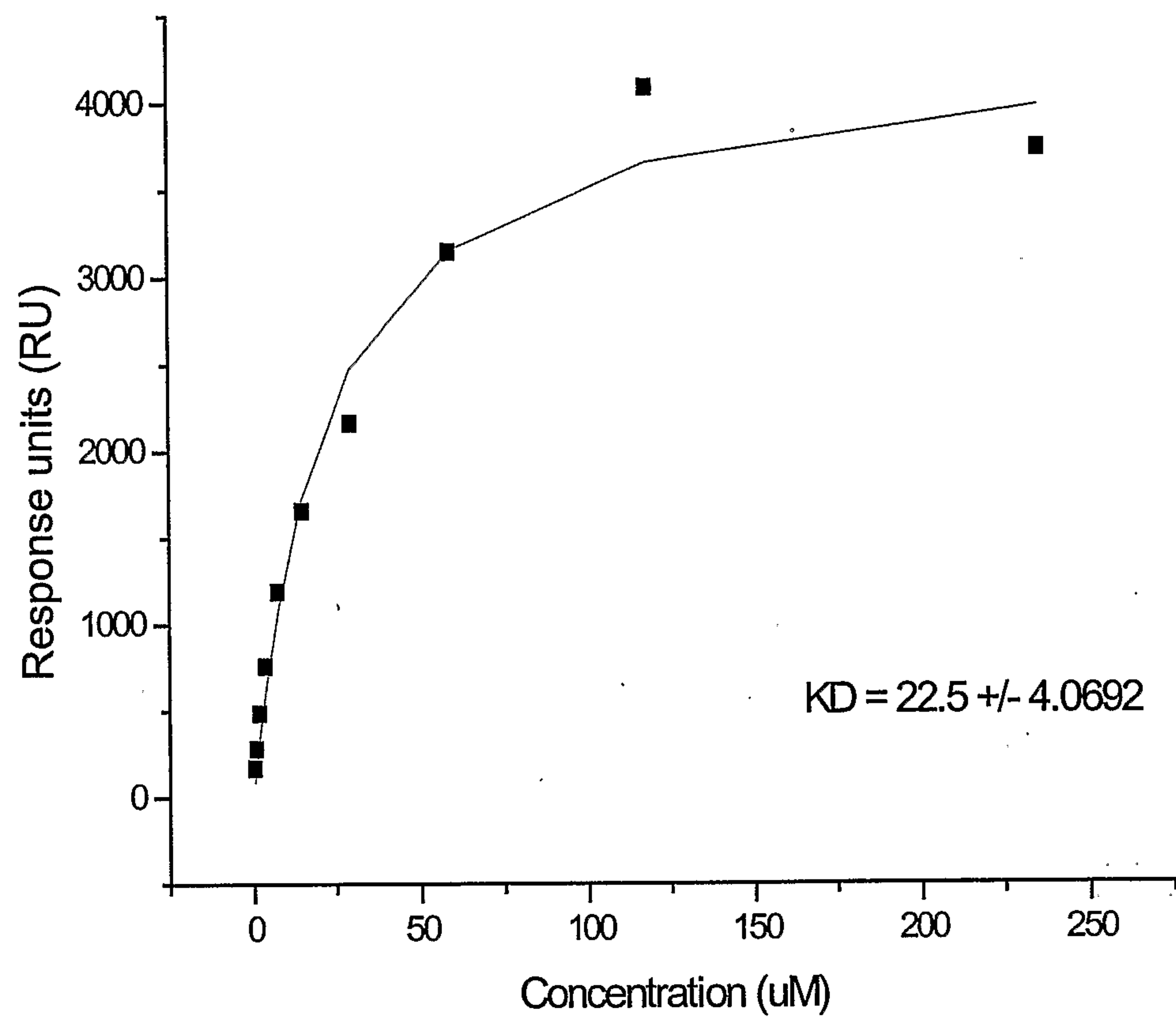
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Figure 31



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Figure 32



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Figure 33a

atgaaggaggtggagcagaattctggacccctcagtggtccagagggagccattg
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acaatattctgggaaaagccctgagttgataatgttcatatactccaatggtgac
aaagaagatggaaggtttacagcacagctcaataaagccagccagtatgtttctc
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aatccagtgacaagtctgtctgcctattcaccgatttttgattctcaaacaatgt
gtcaciaaagtaaggattctgatgtgtatatcacagacaaatgtgtgctagacatg
aggtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgact
ttgcatgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccc
cagcccagaaagttcctaa

Figure 33b

atggggtcatgcagaacccaagacacctgggtcaggaggaggggacaggaggcaa
gactgagatgcagcccaatgaaaggacacagtcattgtttactggtatcggcagct
cccagaggaaggtctgaaattcatgggtttatctccagaaagaaaatatcatagat
gagtcaggaatgccaaaggaacgattttctgctgaatttcccaaagagggcccca
gcatcctgaggatccagcaggttagtgcgaggagattcggcagcttatttctgtgc
cagctcaccacagacaggggggcacagatacgcagtattttggcccaggcaccgg
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atgactccagatacgtctctgagcagccgcctgaggggtctcggccaccttctggca
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Figure 34a

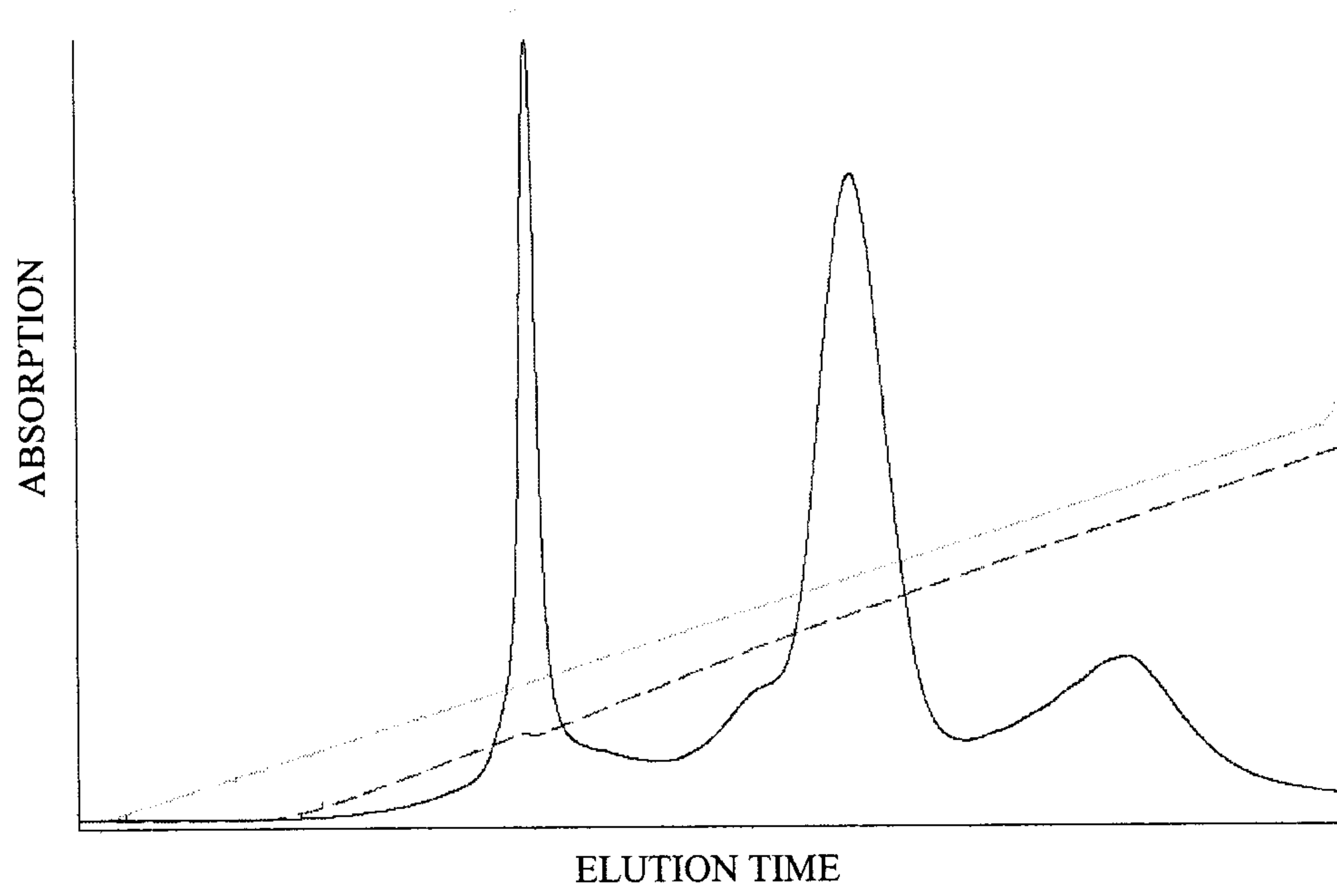
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SDSATYLCVAVKGGSGGYQKVTFGTGTLQVIPNIQNPDPA
VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKC
VLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFF
PSPESS Stop

Figure 34b

MGVMQNPRHLVRRRGQEARLRCSPMKGHSHVYWYRQLP
EEGLKFMVYLQKENIIDESGMPKERFSAEFPKEGPSILRIQ
QVVRGDSAAYFCASSPQTGGTDTQYFGPGTRLTVLEDLKN
VFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSW
WVNGKEVHSGVCTDPQPLKEQPALNDSRYALSSRLRVSAT
FWQDPRNHFRQVQFYGLSENDEWTQDRAKPVTVQIVSAE
AWGRAD Stop

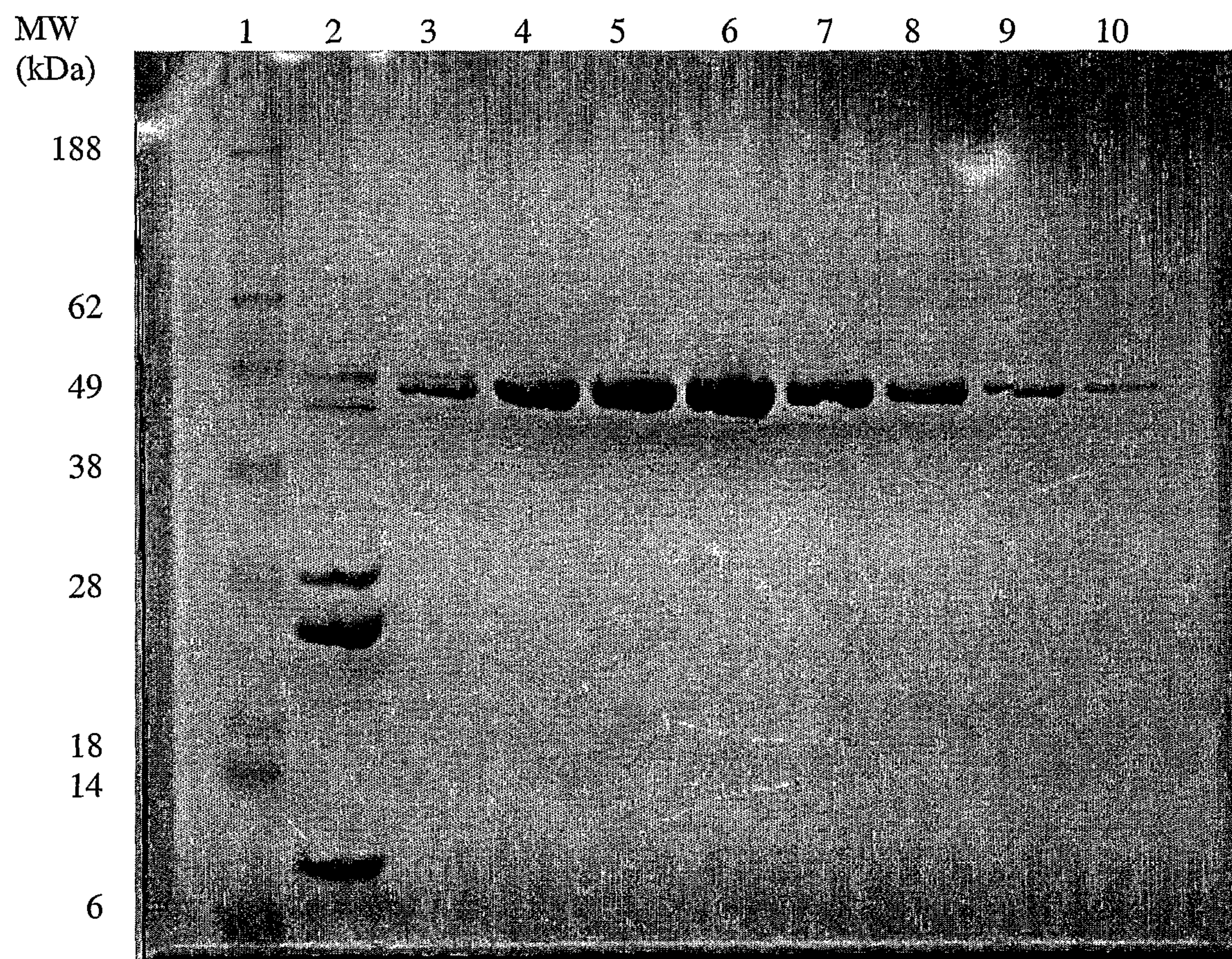
28/81

Figure 35



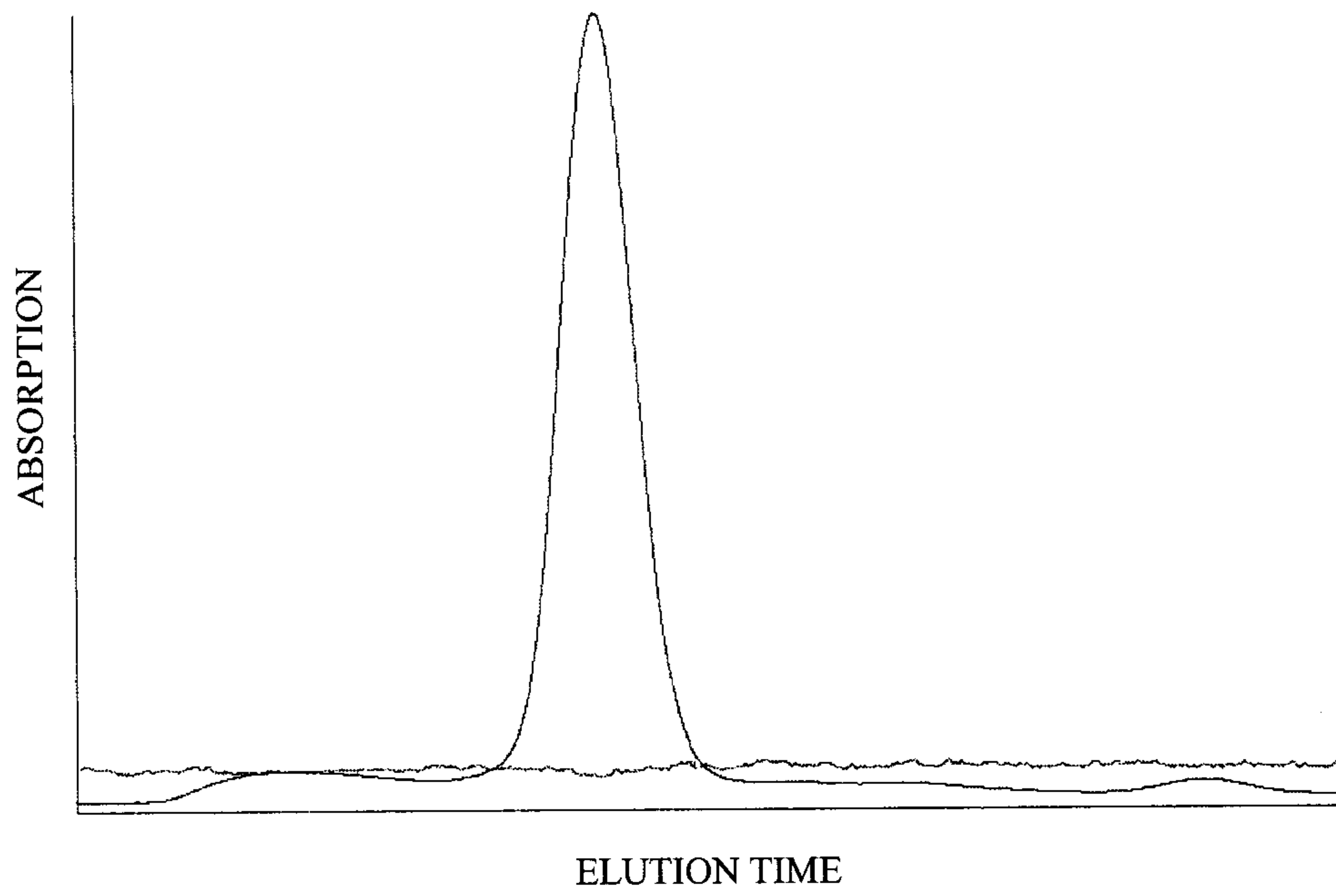
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Figure 37



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Figure 38



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Figure 39a – T48→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtggtccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtgggc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
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 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 ccagaaagttcctaa

Figure 39b – T48→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY SNGDKEDGRF
 TAQLNKASQY VSLIIRDSQP SDSATYLCAY TTDSWGKLQF GAGTQVVVTP DIQNPDPVY
 QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS DVYITDKCVL DMRSMDFKSN SAVAWSNKSD
 FACANAFNNS IIPEDTFFPS PESS*

Figure 40a – T45→C alpha chain DNA Sequence

Atgcagaaggaagtggagcagaactctggacccctcagtggtccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtgggc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatat**tgt**gacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 ccagaaagttcctaa

Figure 40b – T45→C alpha chain Amino Acid Sequence

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY SNGDKEDGRF
 TAQLNKASQY VSLIIRDSQP SDSATYLCAY TTDSWGKLQF GAGTQVVVTP DIQNPDPVY
 QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS DVYICDKTVL DMRSMDFKSN SAVAWSNKSD
 FACANAFNNS IIPEDTFFPS PESS*

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Figure 41a – S61→C alpha chain DNA Sequence

Atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaaggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
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 tctatggacttcaagagcaactgtgctgtggcctggagcaacaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 41b – S61→C alpha chain Amino Acid Sequence

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPAVY QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVYITDKTVL DMRSMDFKSN CAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

Figure 42a – L50→C alpha chain DNA Sequence

Atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaaggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgtgtgacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 42b – L50→C alpha chain Amino Acid Sequence

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPAVY QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVYITDKTVC DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

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Figure 43a – Y10→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatataactccaatggt
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtgagcagctgagagactctaaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 43b – Y10→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPAVC QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVIITDKTVL DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

Figure 44a – S15→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatataactccaatggt
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactgtaaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 44b – S15→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPVY QLRDCKSSDK SVCLFTDFDS QTNVSQSKDS
 DVIITDKTVL DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

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Figure 45a – L12→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatattctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaaggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagtcagagactctaaat
 ccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 45b – L12→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VSLLRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPVY QCRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVYITDKTVL DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

Figure 46a – V22→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatattctgggaaaagccctgagttgataatgtccatatactccaatggg
 gacaaagaagatggaaggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaaat
 ccagtgacaagtctgcctattcaccgattttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 46b – V22→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VSLLRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPVY QLRDSKSSDK SCCLFTDFDS QTNVSQSKDS
 DVYITDKTVL DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

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Figure 47a – M52→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggt
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaaat
 ccagtgacaagtctgtctgcctattcaccgatttttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagact**tgtagg**
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 47b – M52→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPVAV QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVYITDKTVL DCRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

Figure 48a – Y43→C α chain

atgcagaaggaagtggagcagaactctggacccctcagtgttccagagggagcca
 ttgcctctctcaactgcacttacagtgaccgaggttcccagtccttcttctggta
 cagacaatatctctgggaaaagccctgagttgataatgtccatatactccaatggt
 gacaaagaagatggaagggtttacagcacagctcaataaagccagccagtatgttt
 ctctgctcatcagagactcccagcccagtgattcagccacctacctctgtgccgt
 tacaactgacagctgggggaaattgcagtttggagcagggacccaggttgtggtc
 accccagatatccagaaccctgaccctgccgtgtaccagctgagagactctaaat
 ccagtgacaagtctgtctgcctattcaccgatttttgattctcaaacaatgtgtc
 acaaagtaaggattctgatgtgt**tgtagg**atcacagacaaaactgtgctagacatgagg
 tctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttg
 catgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccag
 cccagaaagttcctaa

Figure 48b – Y43→C α chain

MQ

K₁EVEQNSGPL SVPEGAIASL NCTYSDRGSQ SFFWYRQYSG KSPELIMSIY
 SNGDKEDGRF TAQLNKASQY VLLIRDSQP SDSATYLCAV TTDSWGKLQF
 GAGTQVVVTP DIQNPDPVAV QLRDSKSSDK SVCLFTDFDS QTNVSQSKDS
 DVCITDKTVL DMRSMDFKSN SAVAWSNKSD FACANAFNNS IIPEDTFFPS PESS*

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Figure 49a – Ser57→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
at t t c c c g c t c a g g c t g c t g t c g g c t g c t c c c t c c c a g a c a t c t g t g t a c t t c t g
t g c c a g c a g g c c g g g a c t a g c g g g a g g g c g a c c a g a g c a g t a c t t c g g g c c g g g c
a c c a g g c t c a c g g t c a c a g a g g a c c t g a a a a a c g t g t t c c c a c c c g a g g t c g c t g
t g t t t g a g c c a t c a g a a g c a g a g a t c t c c c a c a c c c a a a a g g c c a c a c t g g t g t g
c c t g g c c a c a g g c t t c t a c c c c g a c c a c g t g g a g c t g a g c t g g t g g g t g a a t g g g
a a g g a g g t g c a c a g t g g g g t c t g c a c a g a c c c g c a g c c c c t c a a g g a g c a g c c c g
c c c t c a a t g a c t c c a g a t a c g c t c t g a g c a g c c g c c t g a g g g t c t c g g c c a c c t t
c t g g c a g g a c c c c c g c a a c c a c t t c c g c t g t c a a g t c c a g t t c t a c g g g c t c t c g
g a g a a t g a c g a g t g g a c c c a g g a t a g g g c c a a a c c c g t c a c c c a g a t c g t c a g c g
c c g a g g c c t g g g g t a g a g c a g a c t a a

Figure 49b Ser57→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
FYPDHVELSW WVNGKEVHSG VCTDPQPLKE QPALNDSRYA LSSRLRVSAT
FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

Figure 50a – Ser77→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
a t t t c c c g c t c a g g c t g c t g t c g g c t g c t c c c t c c c a g a c a t c t g t g t a c t t c t g
t g c c a g c a g g c c g g g a c t a g c g g g a g g g c g a c c a g a g c a g t a c t t c g g g c c g g g c
a c c a g g c t c a c g g t c a c a g a g g a c c t g a a a a a c g t g t t c c c a c c c g a g g t c g c t g
t g t t t g a g c c a t c a g a a g c a g a g a t c t c c c a c a c c c a a a a g g c c a c a c t g g t g t g
c c t g g c c a c a g g c t t c t a c c c c g a c c a c g t g g a g c t g a g c t g g t g g g t g a a t g g g
a a g g a g g t g c a c a g t g g g g t c a g c a c a g a c c c g c a g c c c c t c a a g g a g c a g c c c g
c c c t c a a t g a c t c c a g a t a c g c t c t g t g a g c c g c c t g a g g g t c t c g g c c a c c t t
c t g g c a g g a c c c c c g c a a c c a c t t c c g c t g t c a a g t c c a g t t c t a c g g g c t c t c g
g a g a a t g a c g a g t g g a c c c a g g a t a g g g c c a a a c c c g t c a c c c a g a t c g t c a g c g
c c g a g g c c t g g g g t a g a g c a g a c t a a

Figure 50b Ser77→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
FYPDHVELSW WVNGKEVHSG VSTDPOPLKE QPALNDSRYA LCSRLRVSAT
FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

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Figure 51a – Ser17→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggctcctgaagacaggacaga
 gcatgacactgcagtgtgccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtagtacttcgggcccgggc
 accaggctcacgggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtttgagccatggaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagacccgcagccccctcaaggagcagccccg
 ccctcaatgactccagatacgctctgagcagccgcctgagggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggaaccaggatagggccaaacccgtcacccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 51b Ser17→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PCEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDPOPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

Figure 52a – Val 13→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggctcctgaagacaggacaga
 gcatgacactgcagtgtgccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtagtacttcgggcccgggc
 accaggctcacgggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 gttttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagacccgcagccccctcaaggagcagccccg
 ccctcaatgactccagatacgctctgagcagccgcctgagggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggaaccaggatagggccaaacccgtcacccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 52 Val 13→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVACFE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDPOPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

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Figure 53a – Asp 59→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
 gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgcctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtagtctcgggccgggc
 accaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacatgcccgcagccccctcaaggagcagccccg
 ccctcaatgactccagatacgctctgagcagccgcctgagggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggaccaggatagggccaaacccgtcaccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 53b Asp 59→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTCPQPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

Figure 54a – Arg 79→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
 gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgcctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtagtctcgggccgggc
 accaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagaccgcagccccctcaaggagcagccccg
 ccctcaatgactccagatacgctctgagcagcctgaggggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggaccaggatagggccaaacccgtcaccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 54b Arg 79→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDQPLKE QPALNDSRYA LSSCLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

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Figure 55a – Phe 14→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
 gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtacttcgggcccgggc
 accaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtgtgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagacccgcagcccctcaaggagcagcccg
 ccctcaatgactccagatacgctctgagcagccgcctgagggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggacccaggatagggccaaacccgtcaccacagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 55b Phe 14→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPQSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVCE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDPOPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

Figure 56a – Gly 55→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
 gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtacttcgggcccgggc
 accaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgtgtcagcacagacccgcagcccctcaaggagcagcccg
 ccctcaatgactccagatacgctctgagcagccgcctgagggtctcggccacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggacccaggatagggccaaacccgtcaccacagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 56b Gly 55→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPQSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSC VSTDPOPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

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Figure 57a – Leu 63→C β chain

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacaga
 gcatgacactgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcg
 acaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatc
 actgaccaaggagaagtccccaatggctacaatgtctccagatcaaccacagagg
 atttcccgctcaggctgctgtcggctgctccctcccagacatctgtgtacttctg
 tgccagcaggccgggactagcgggagggcgaccagagcagtacttcgggcccgggc
 accaggctcacggtcacagaggacctgaaaaacgtgttcccacccgaggctcgctg
 tgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtg
 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagacccgcagcccctgcaaggagcagcccg
 ccctcaatgactccagatacgctctgagcagccgcctgaggggtctcgggcacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggacccaggatagggccaaacccgtcaccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 57b Leu 63→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFE PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDPOPKCE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

Figure 58a – Glu 15→C β chain

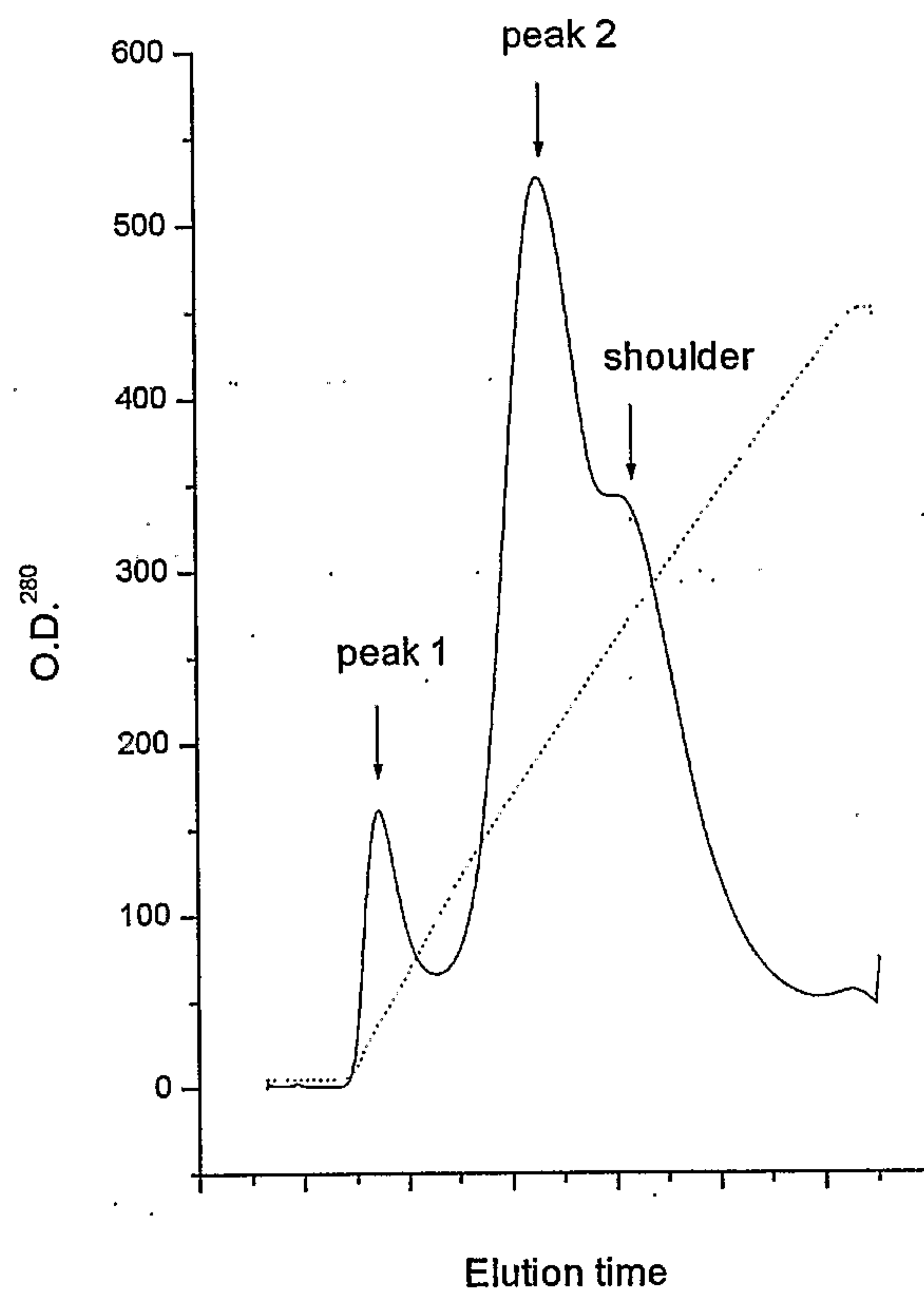
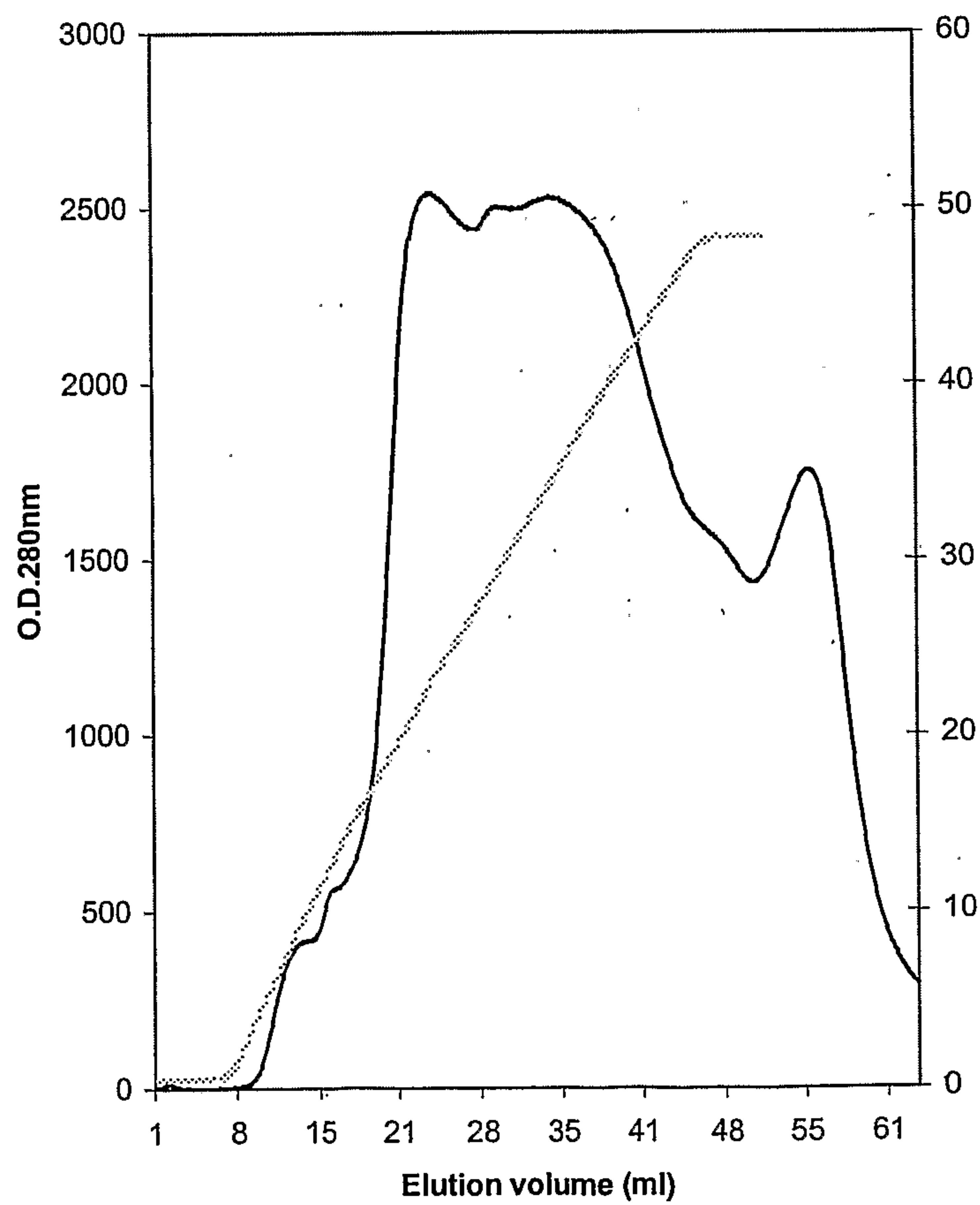
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 cctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatggg
 aaggaggtgcacagtgggggtcagcacagacccgcagcccctcaaggagcagcccg
 ccctcaatgactccagatacgctctgagcagccgcctgaggggtctcgggcacctt
 ctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcg
 gagaatgacgagtggacccaggatagggccaaacccgtcaccagatcgtcagcg
 ccgaggcctggggtagagcagactaa

Figure 58b Glu 15→C β chain

M

N₁AGVTQTPKF QVLKTGQSMT LQCAQDMNHE YMSWYRQDPG MGLRLIHYSV
 GAGITDQGEV PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASRPGLAGG
 RPEQYFGPGT RLTVTEDLKN VFPPEVAVFC PSEAEISHTQ KATLVCLATG
 FYPDHVELSW WVNGKEVHSG VSTDPOPLKE QPALNDSRYA LSSRLRVSAT
 FWQDPRNHFR CQVQFYGLSE NDEWTQDRAK PVTQIVSAEA WGRAD*

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**Figure 59: AE column Thr48/Ser57****Figure 60: AE column Thr45/Ser77**

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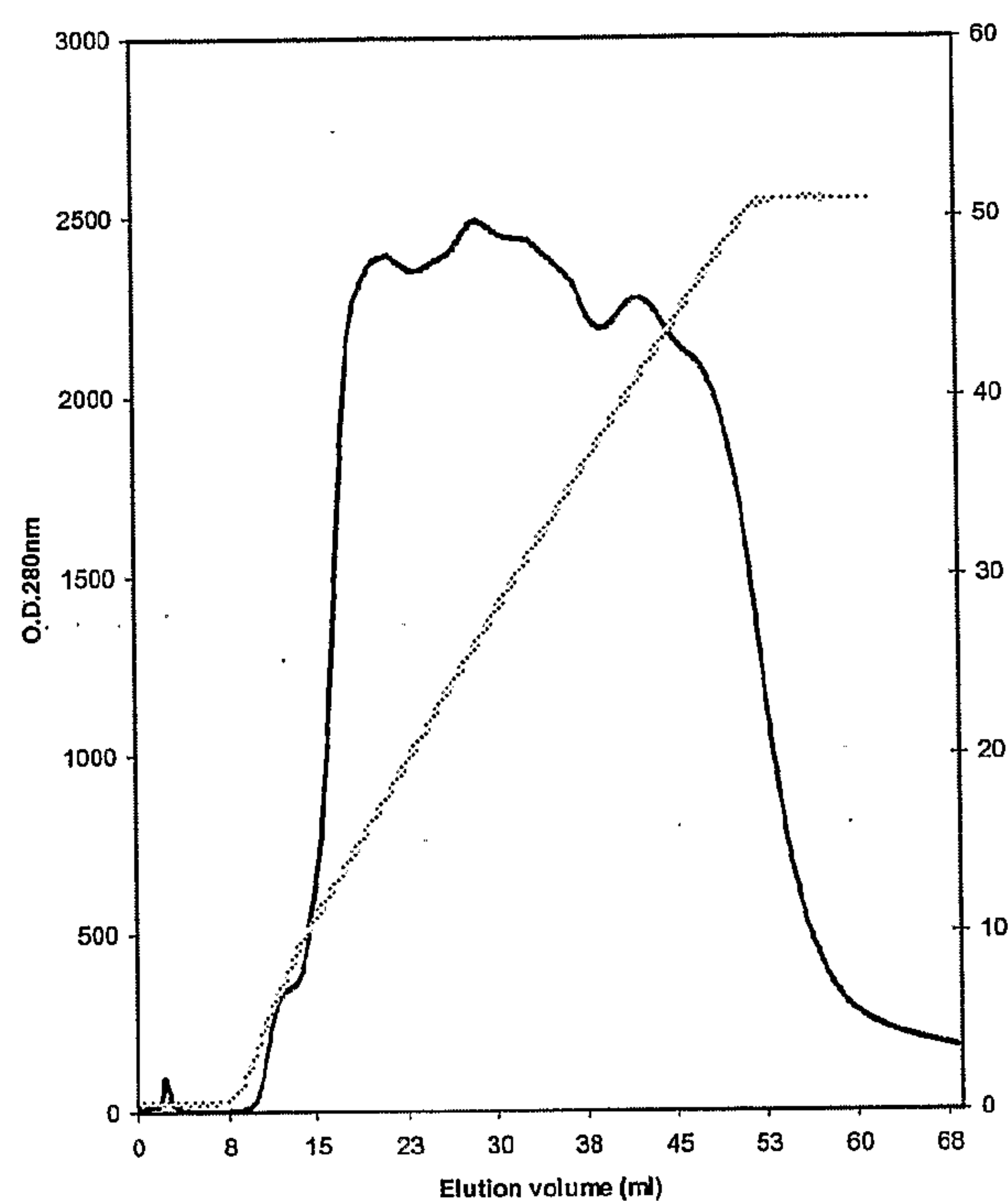


Figure 61: AE column Tyr10/Ser17

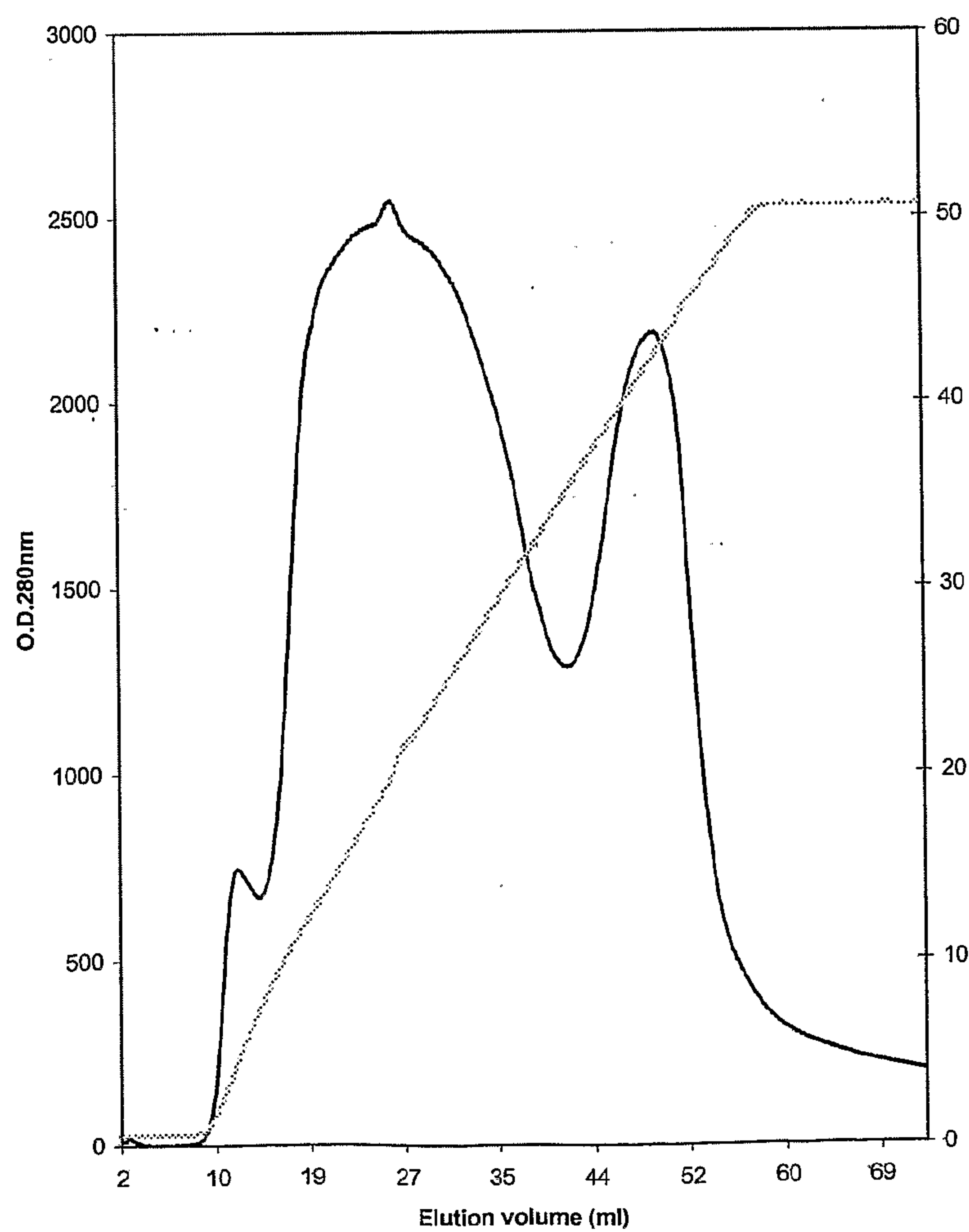


Figure 62: AE column Thr45/Asp59

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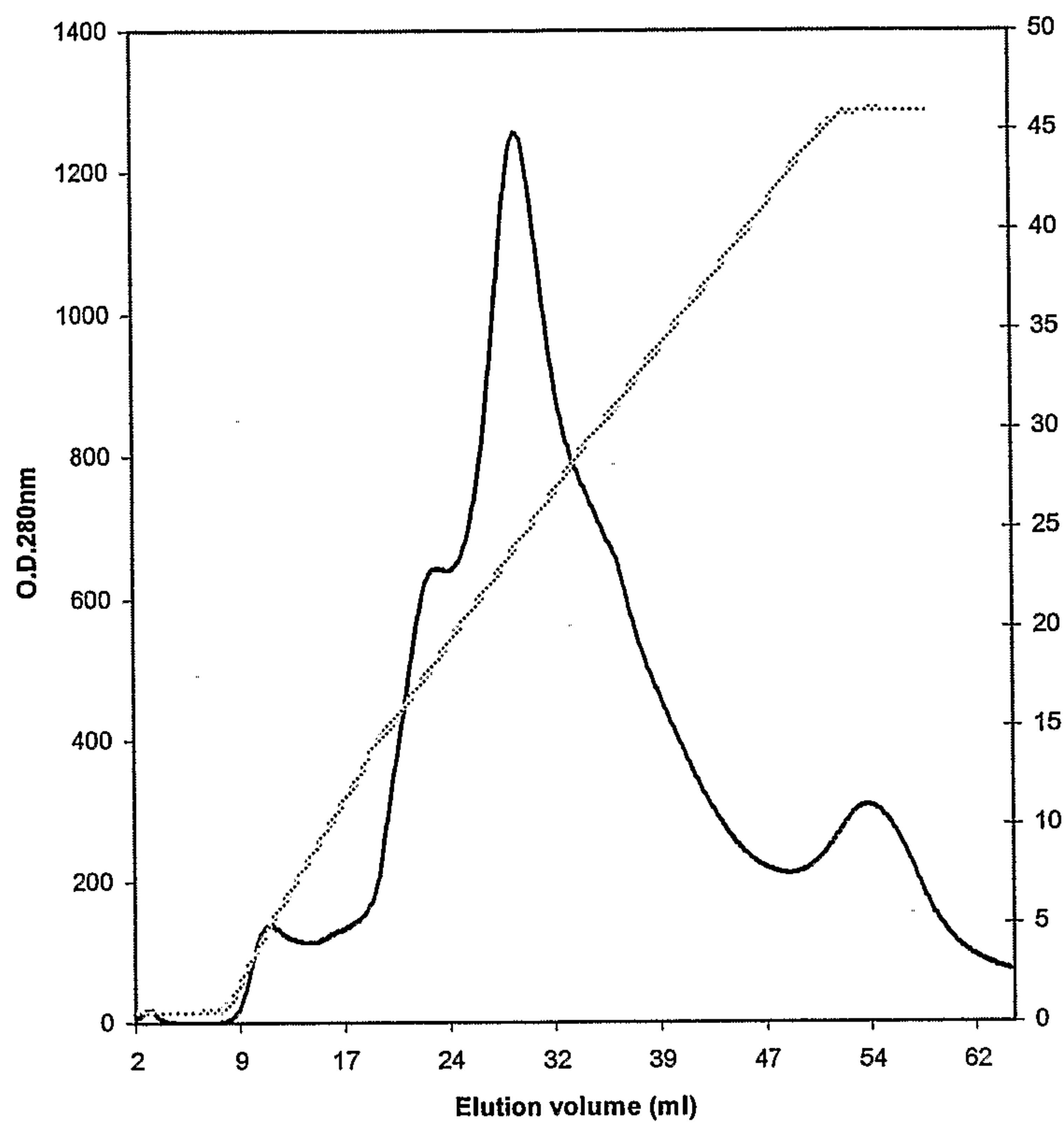


Figure 63: AE column Met52/Gly55

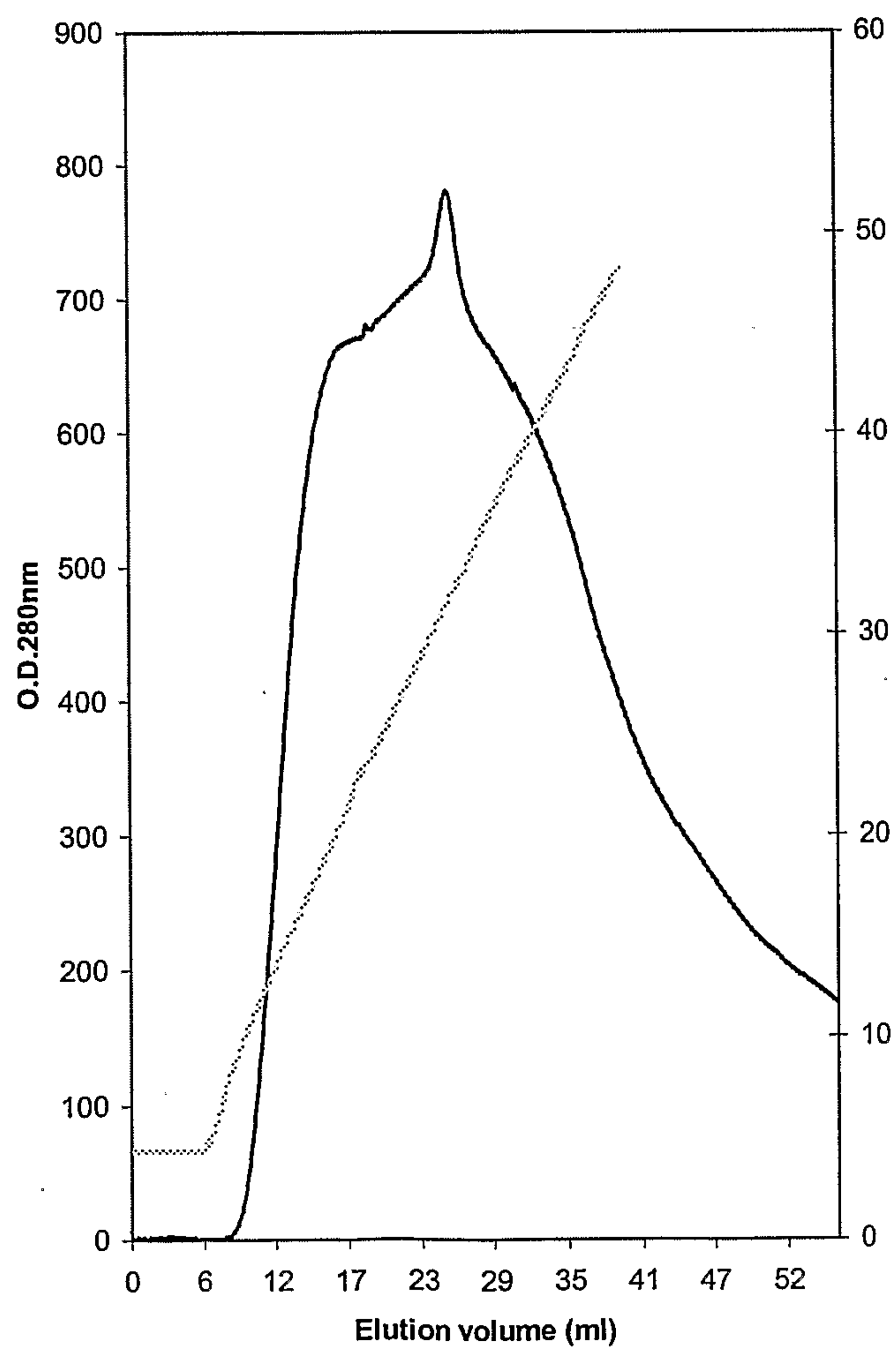


Figure 64: AE column Ser15/Glu15

45/81

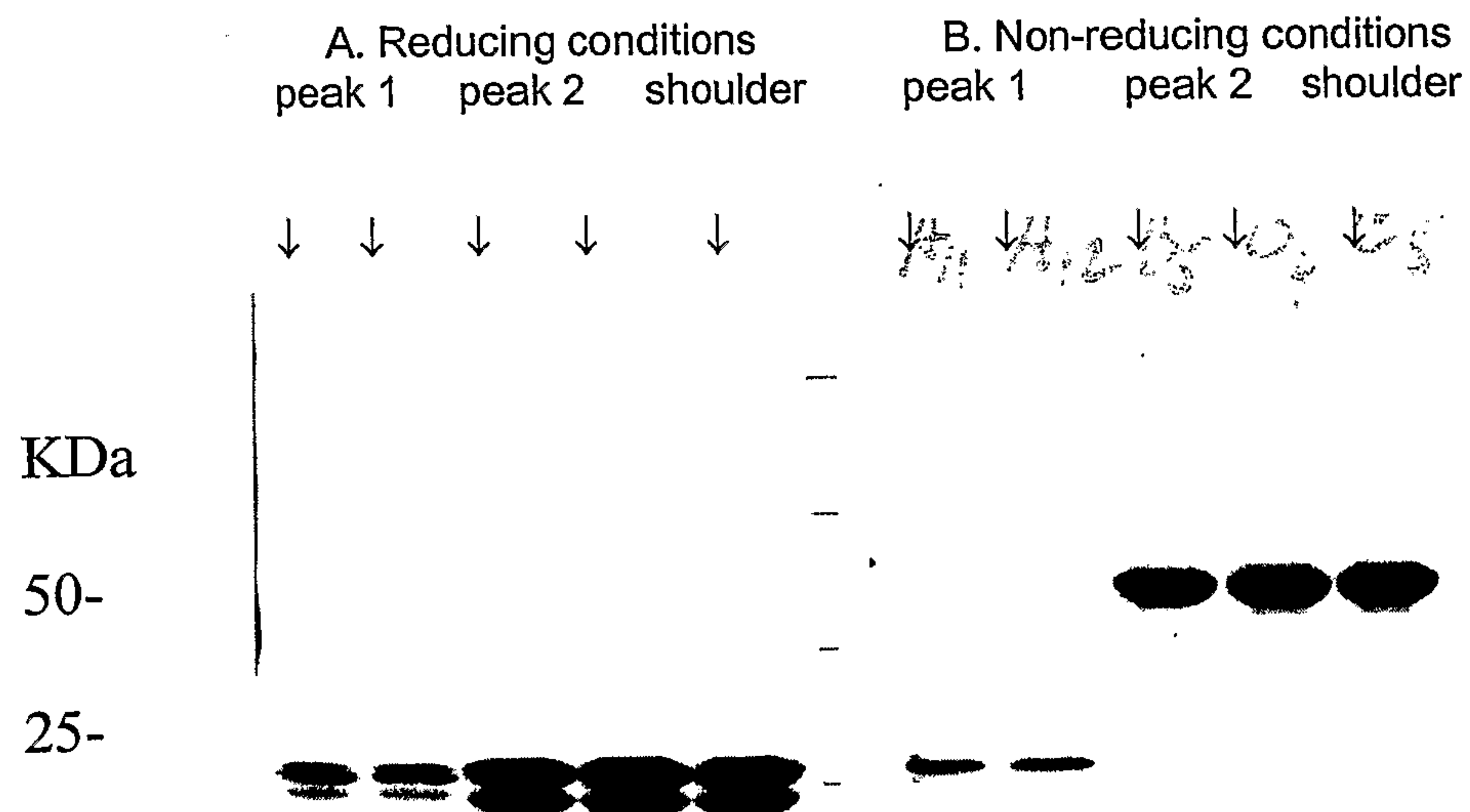


Figure 65: SDS PAGE Thr 48 / Ser 57

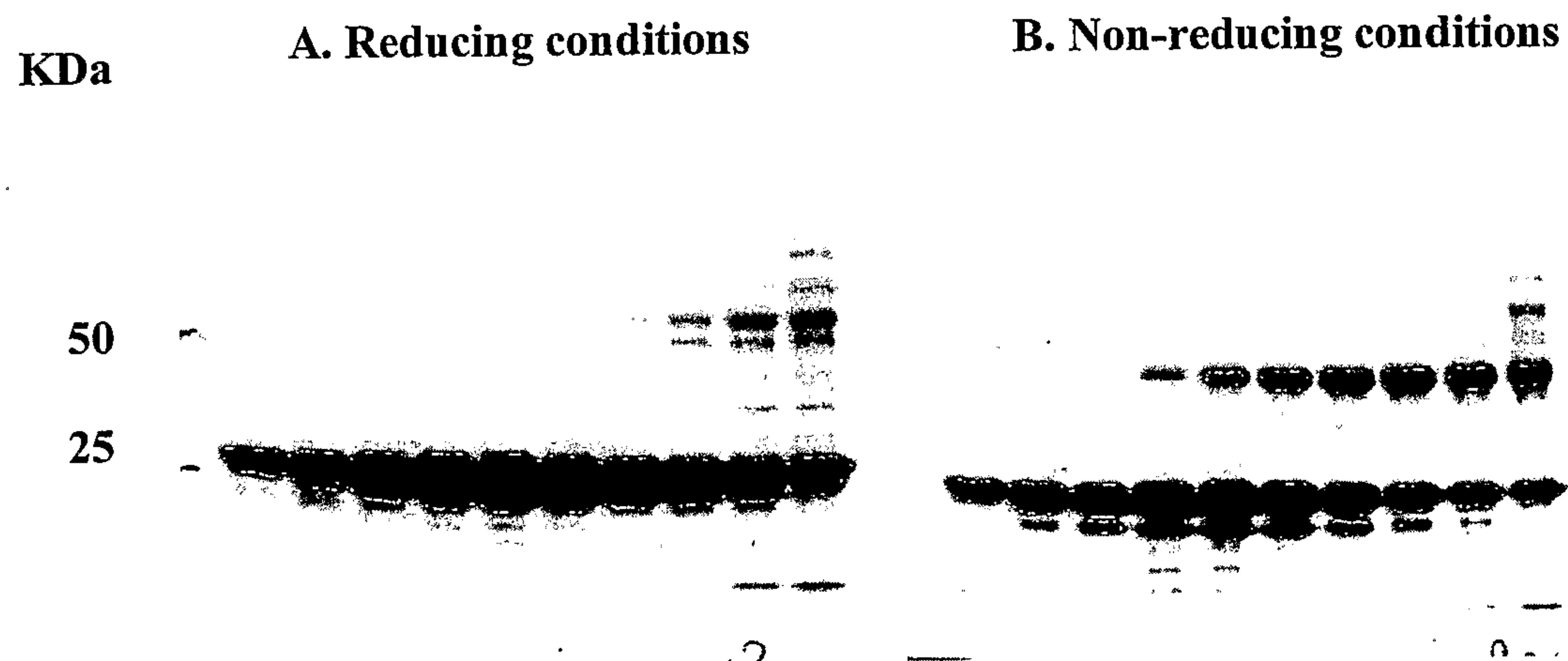
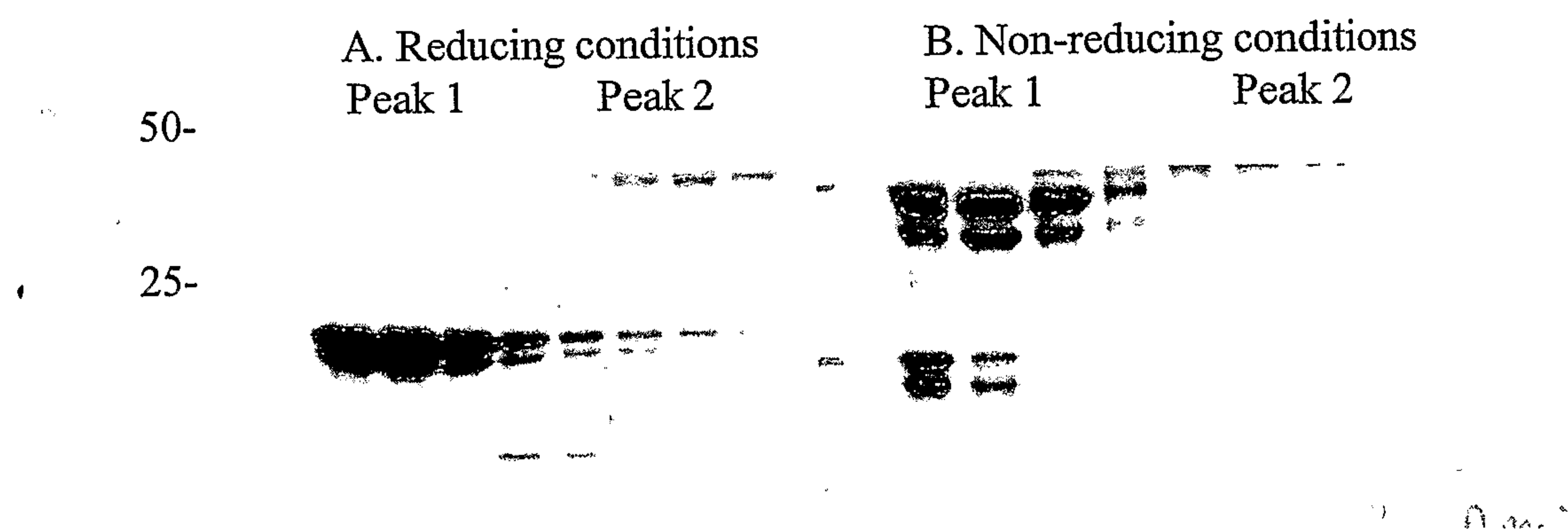
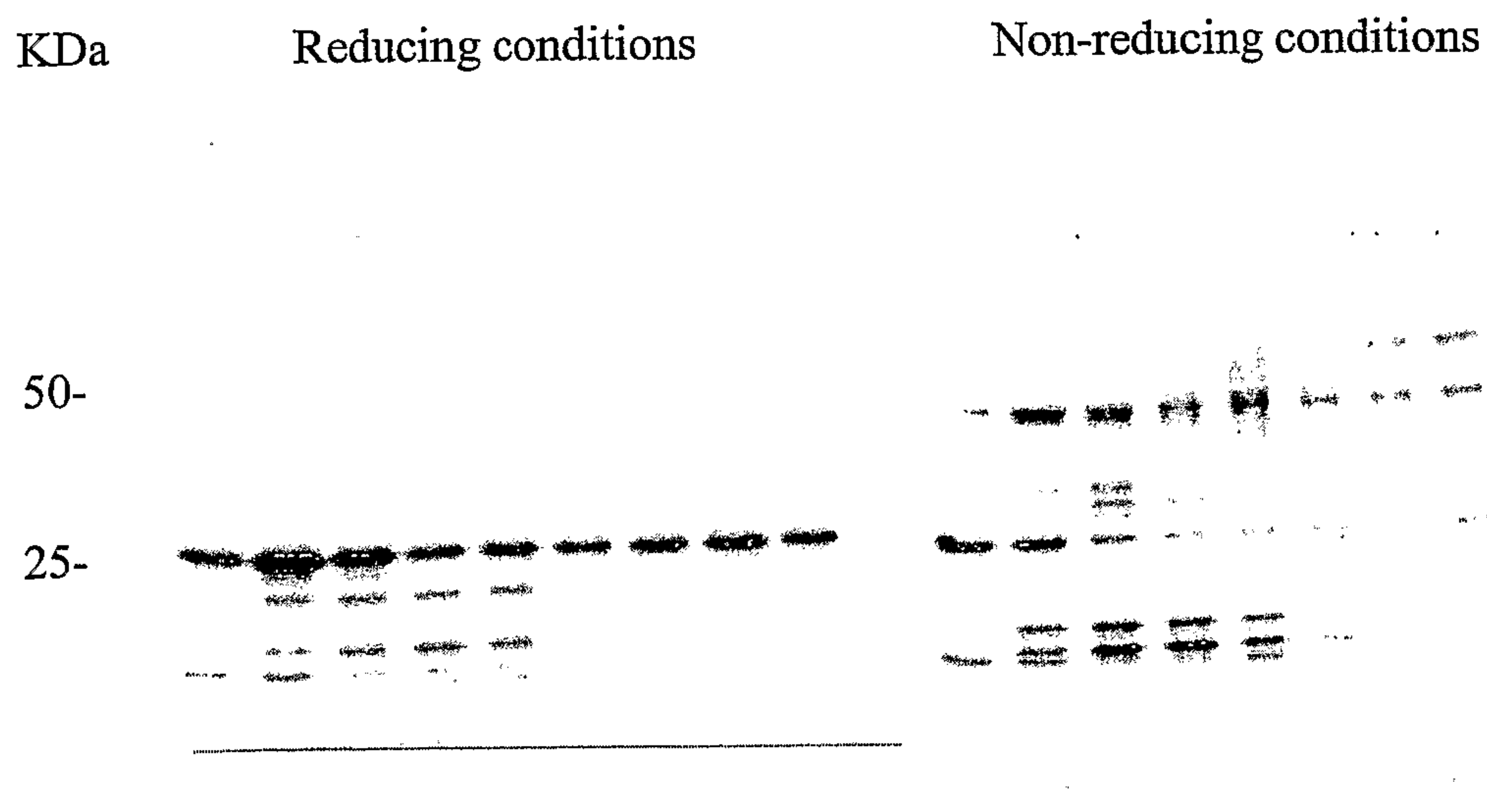


Figure 66: SDS PAGE Thr 45 / Ser 77

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**Figure 67 SDS PAGE Tyr 10 / Ser 17****Figure 68 SDS PAGE Thr 45 / Asp 59**

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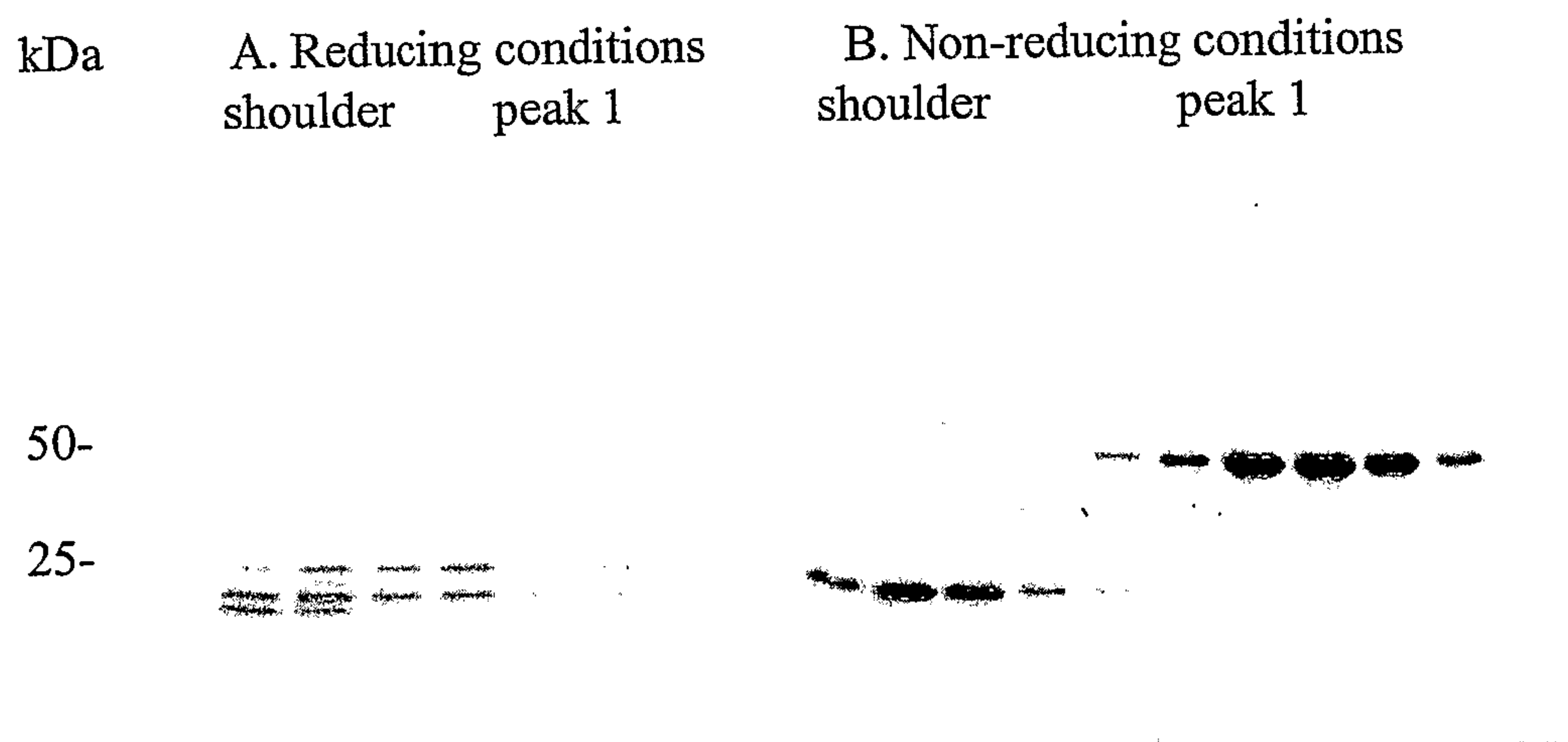


Figure 69: SDS PAGE Met52/Gly55

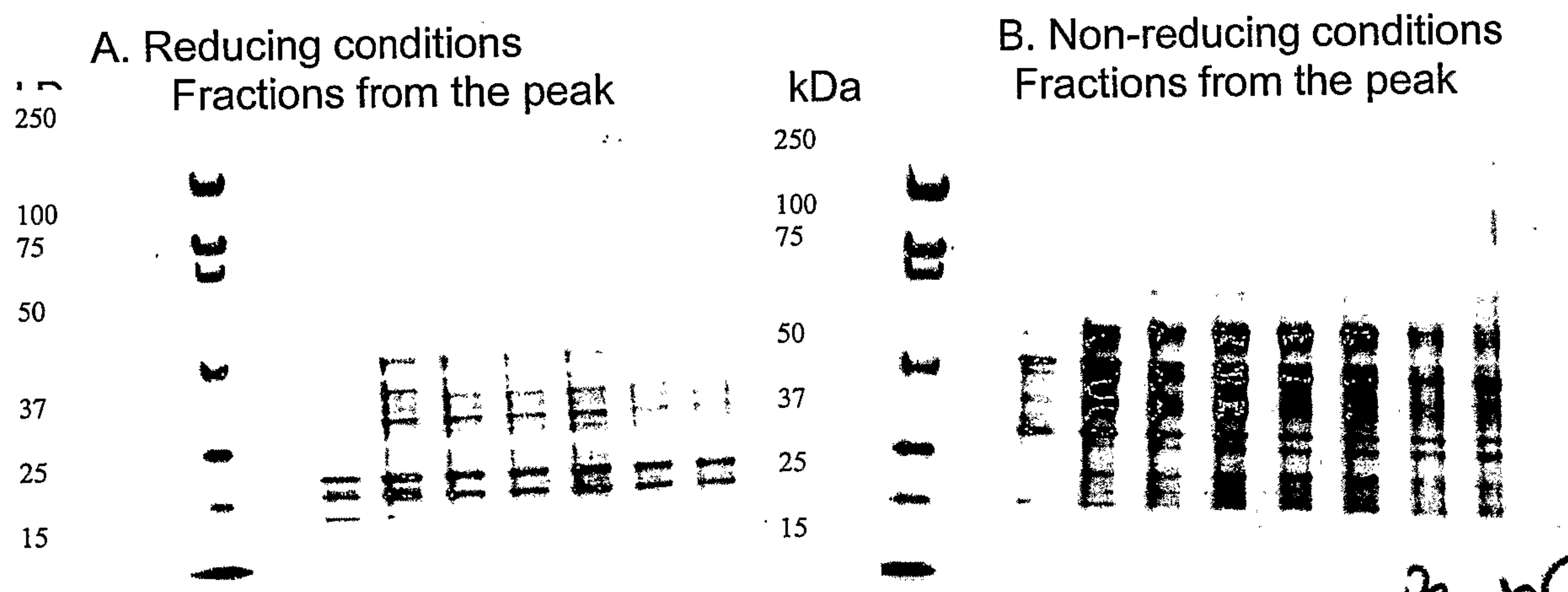
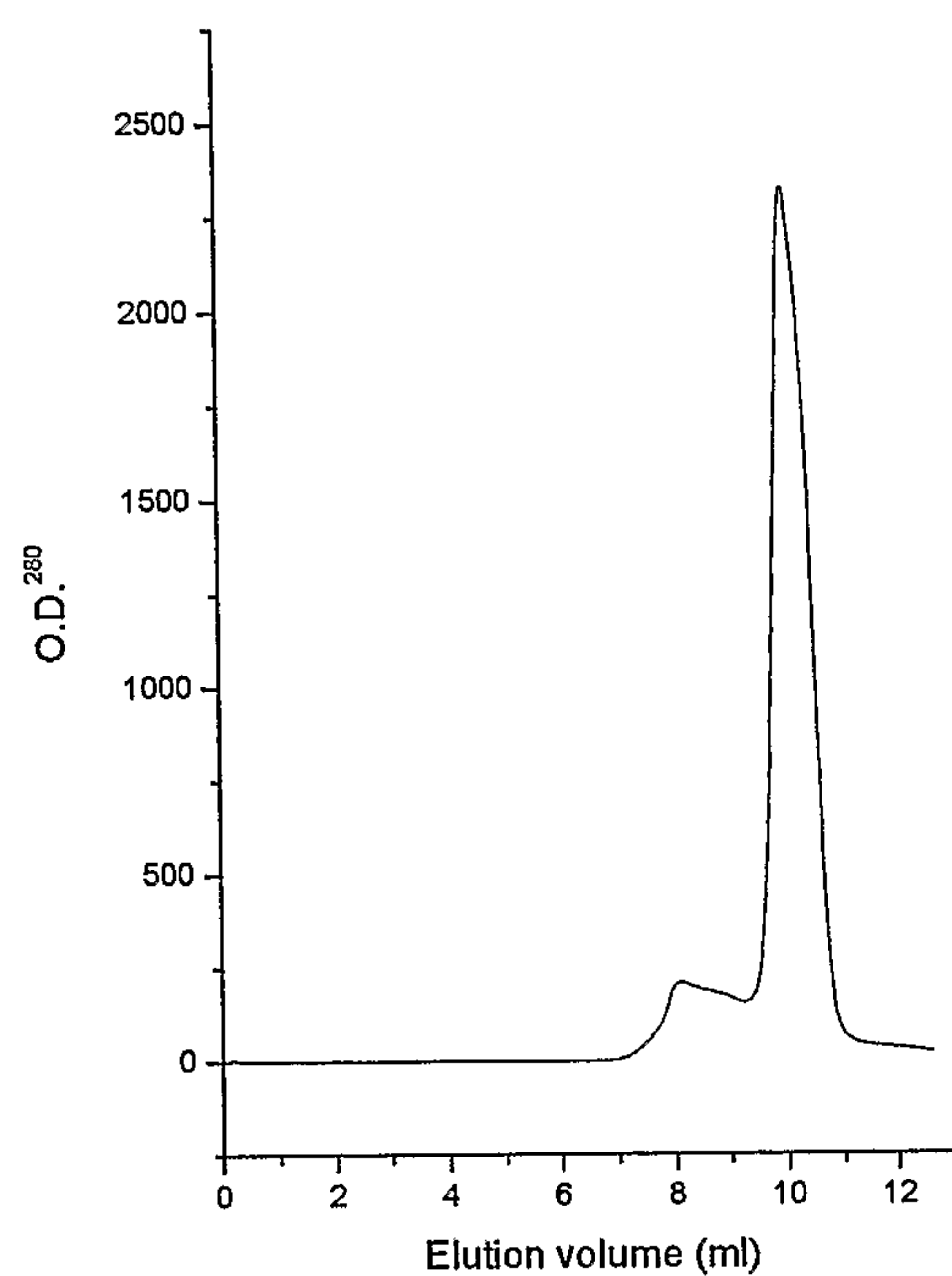
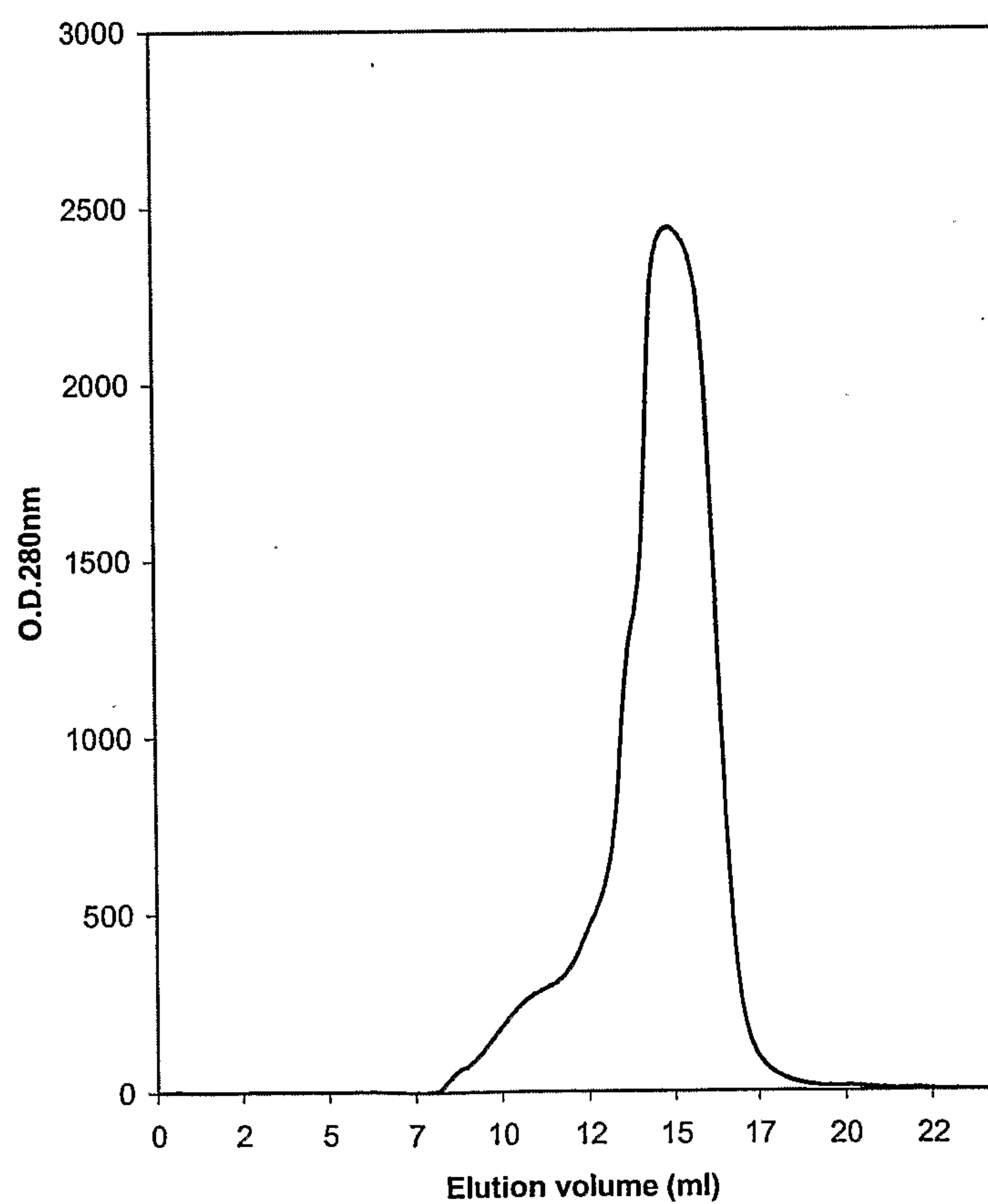
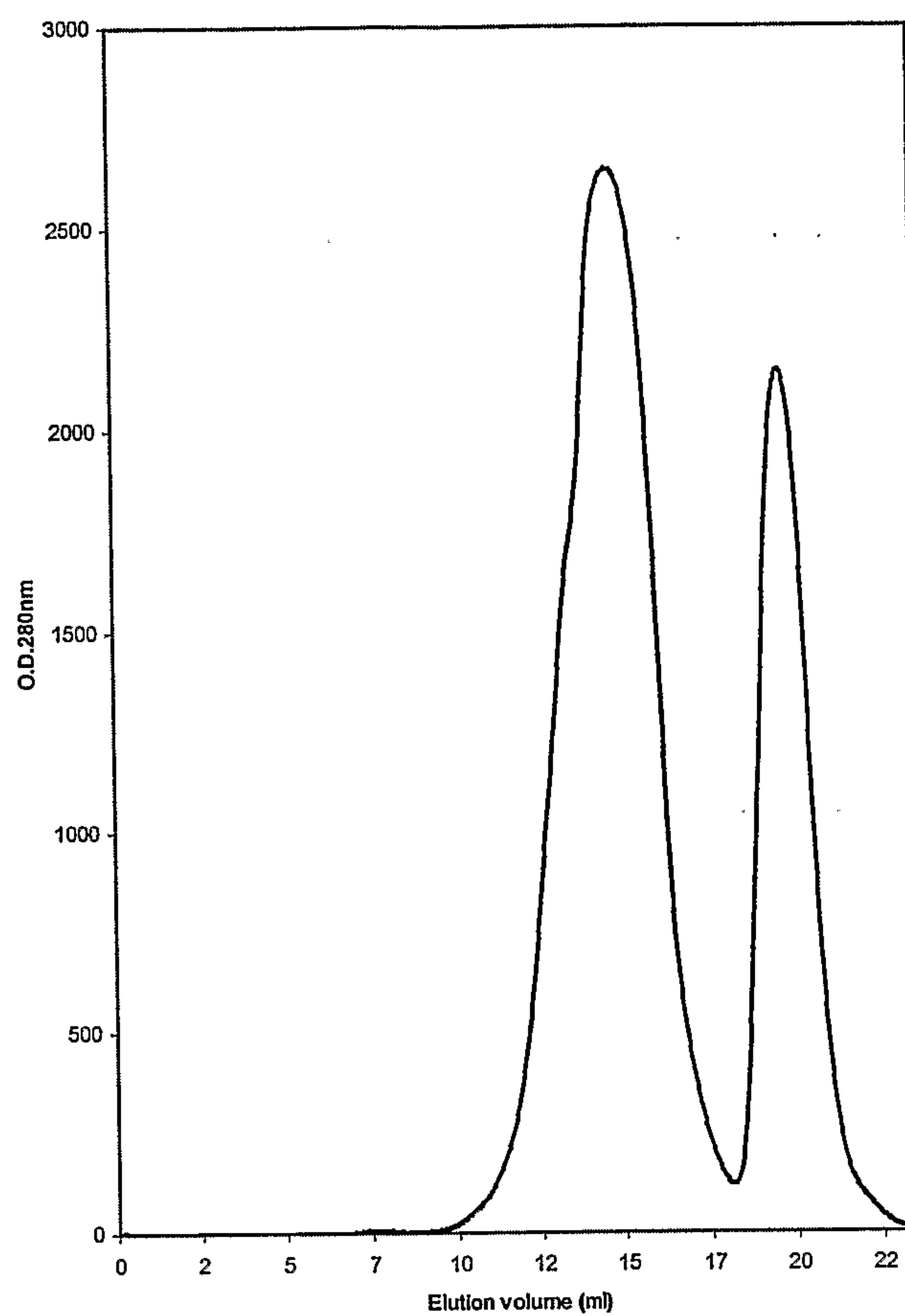
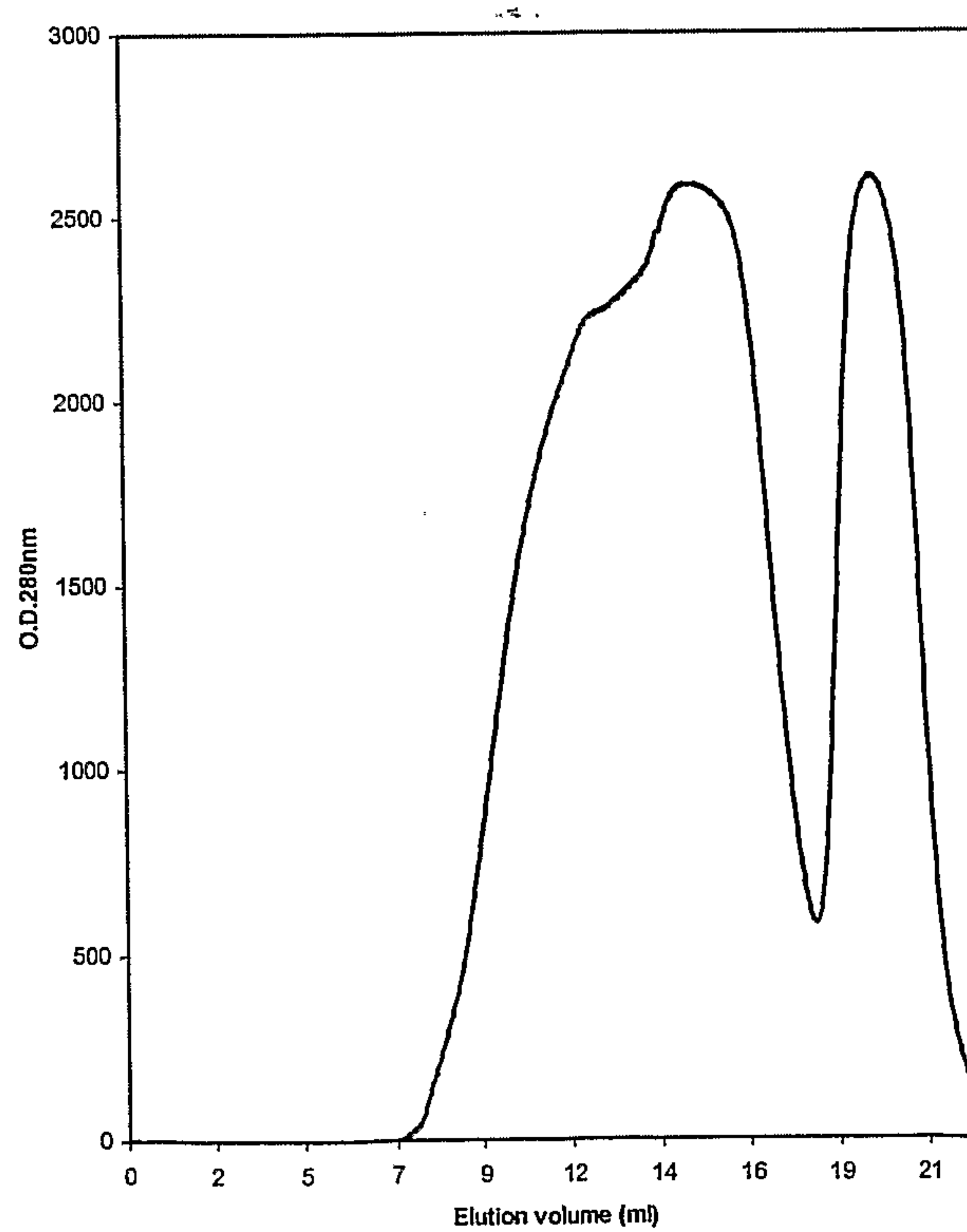


Figure 70: SDS PAGE Ser 15/Glu 15

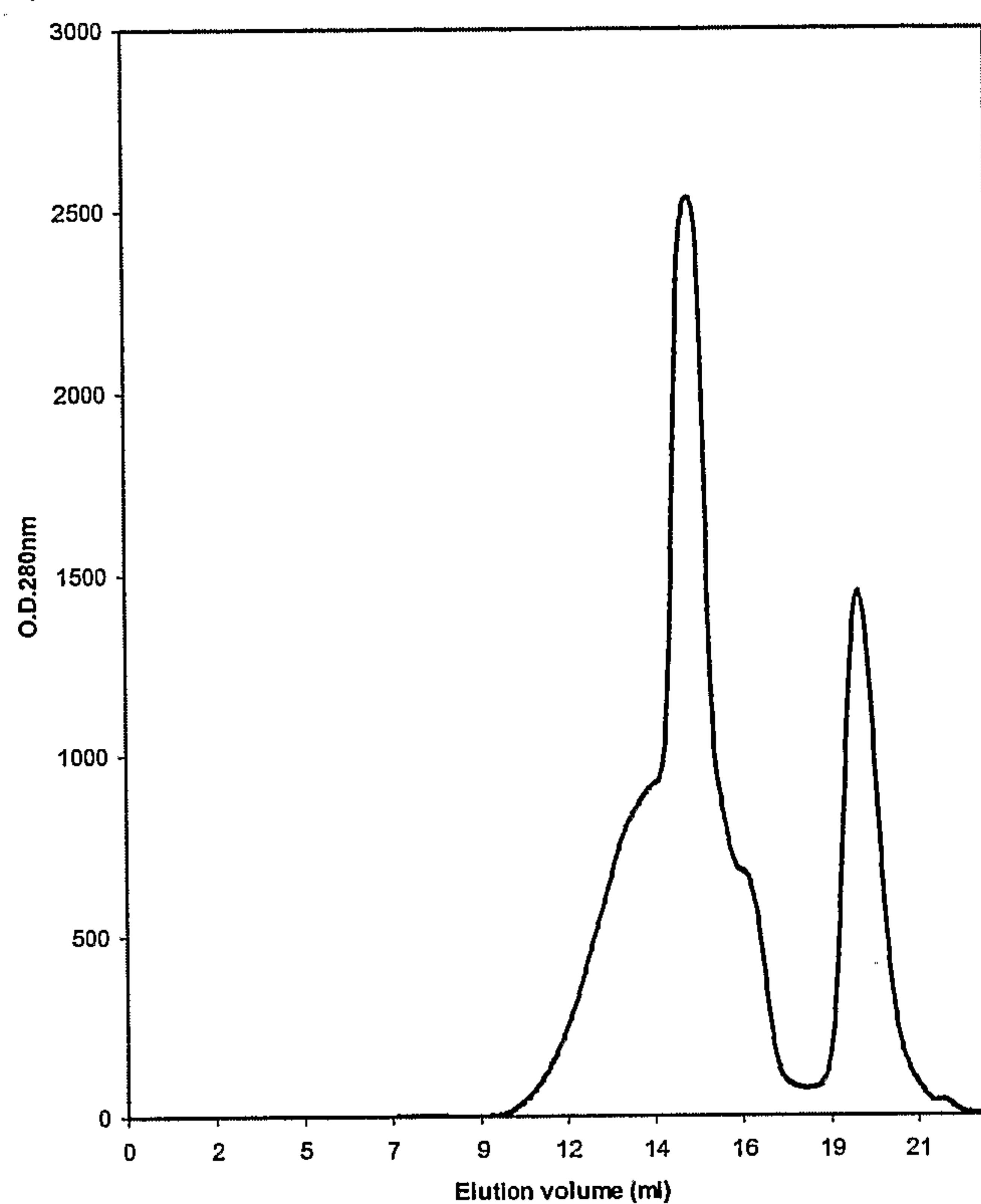
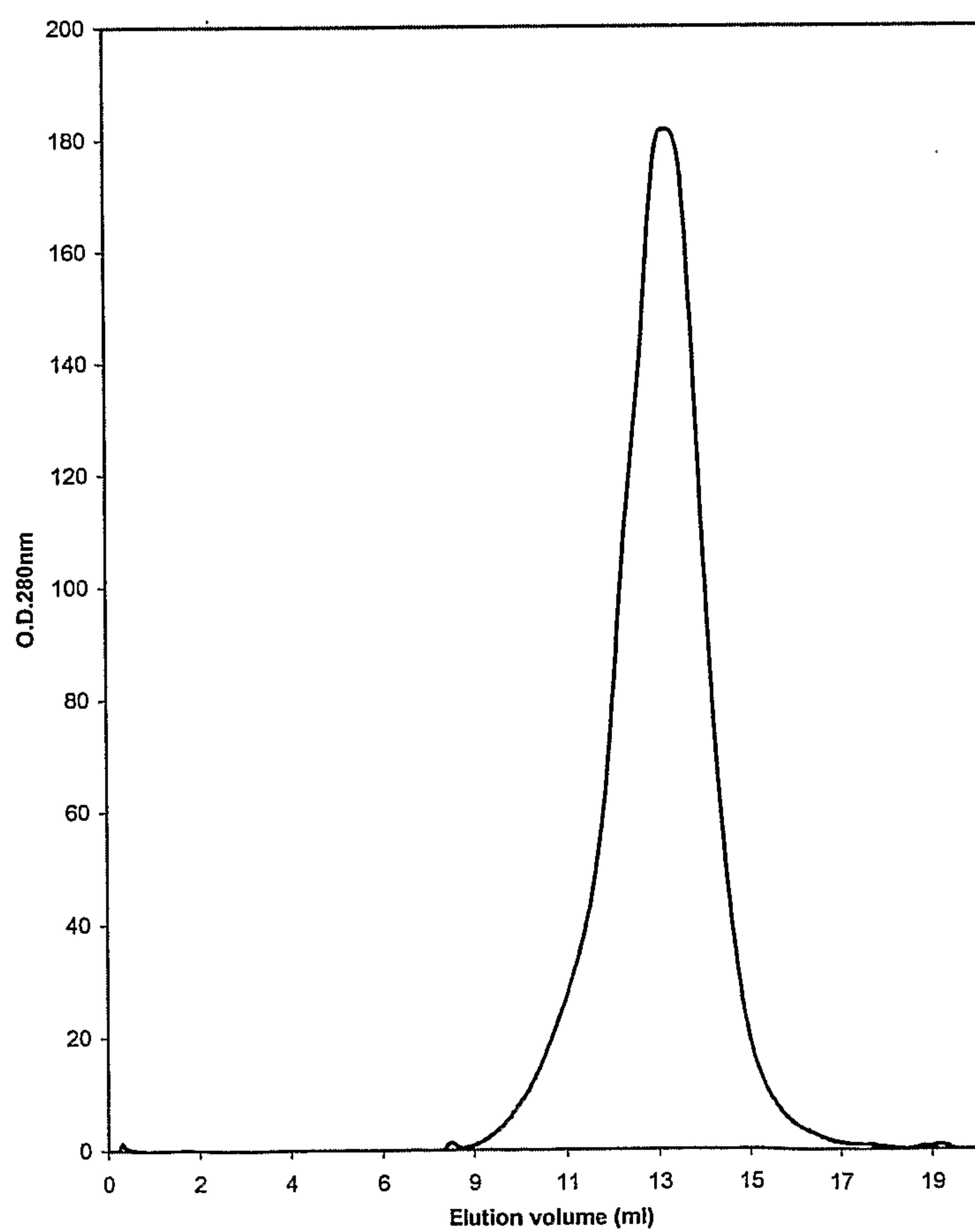
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**Figure 71: SEC Thr48/Ser57****Figure 72: SEC Thr45/Ser77 (200 HR column)**

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**Figure 73: SEC Tyr 10 / Ser 17 (200 HR Column)****Figure 74: SEC Thr 45/Asp 59 (200 HR Column)**

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**Figure 75 SEC Met 52 / Gly 55 (200 HR Column)****Figure 76: SEC Ser 15/Glu 15 (200 HR Column)**

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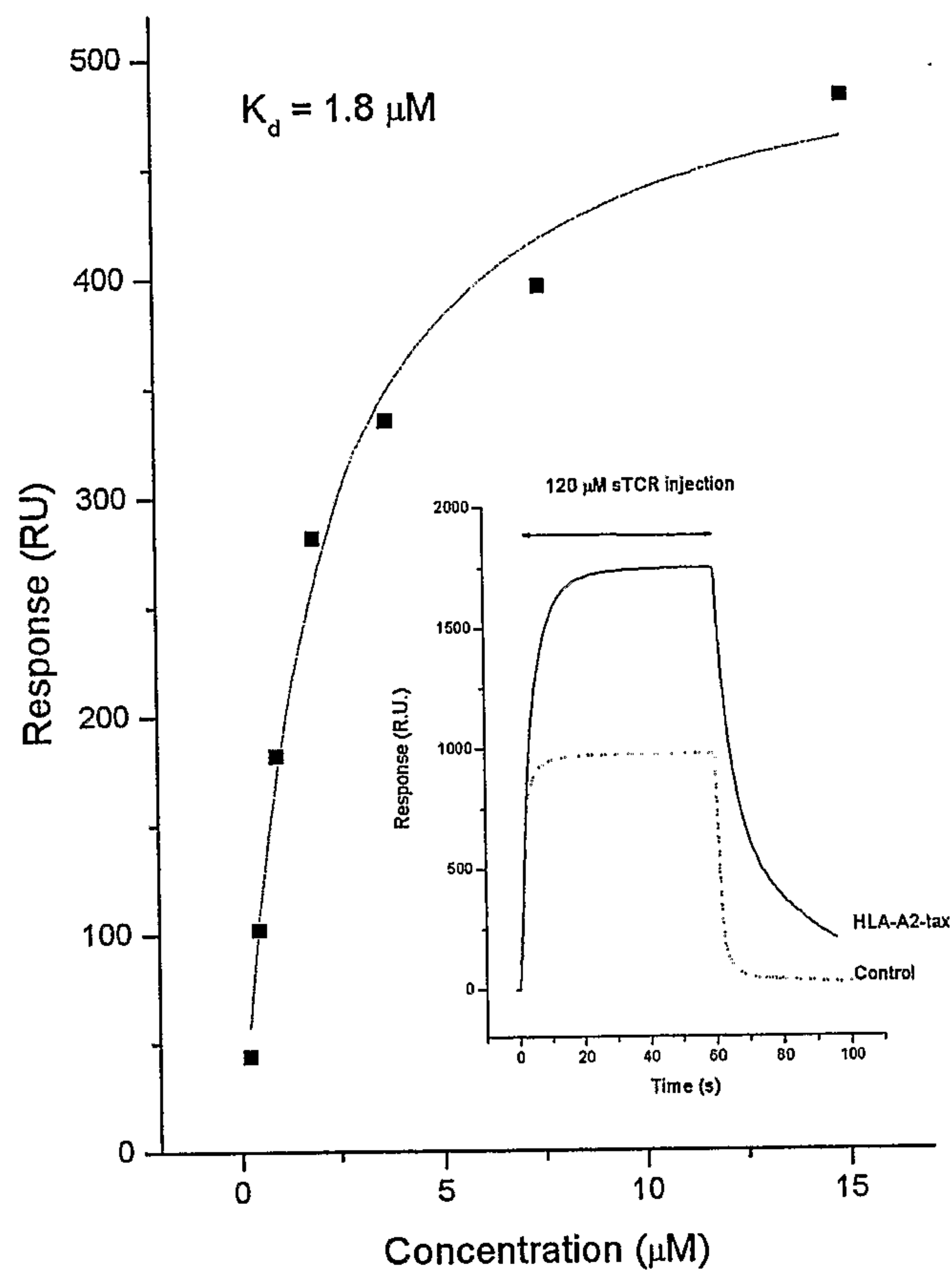


Figure 77: Thr 48/Ser 57

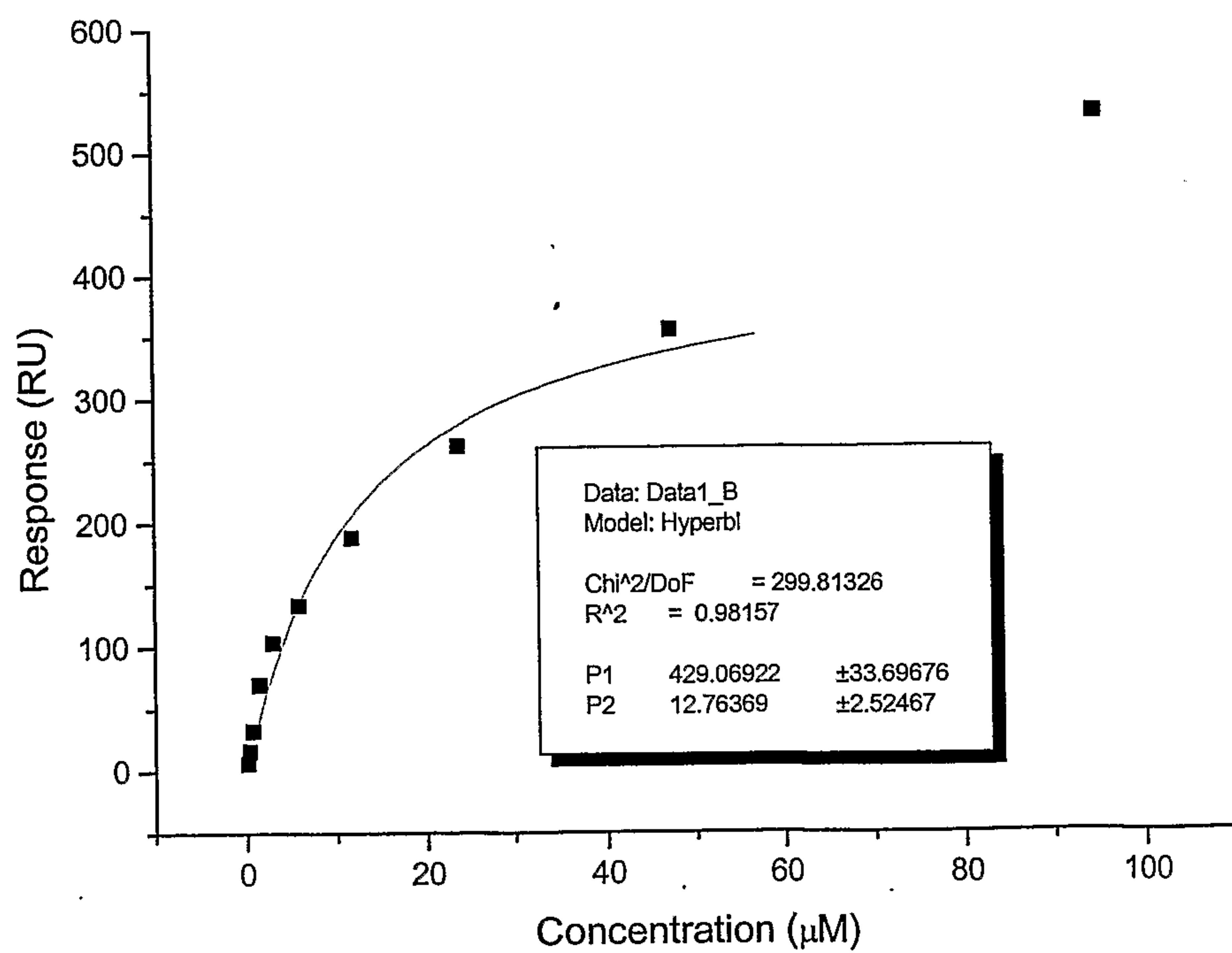
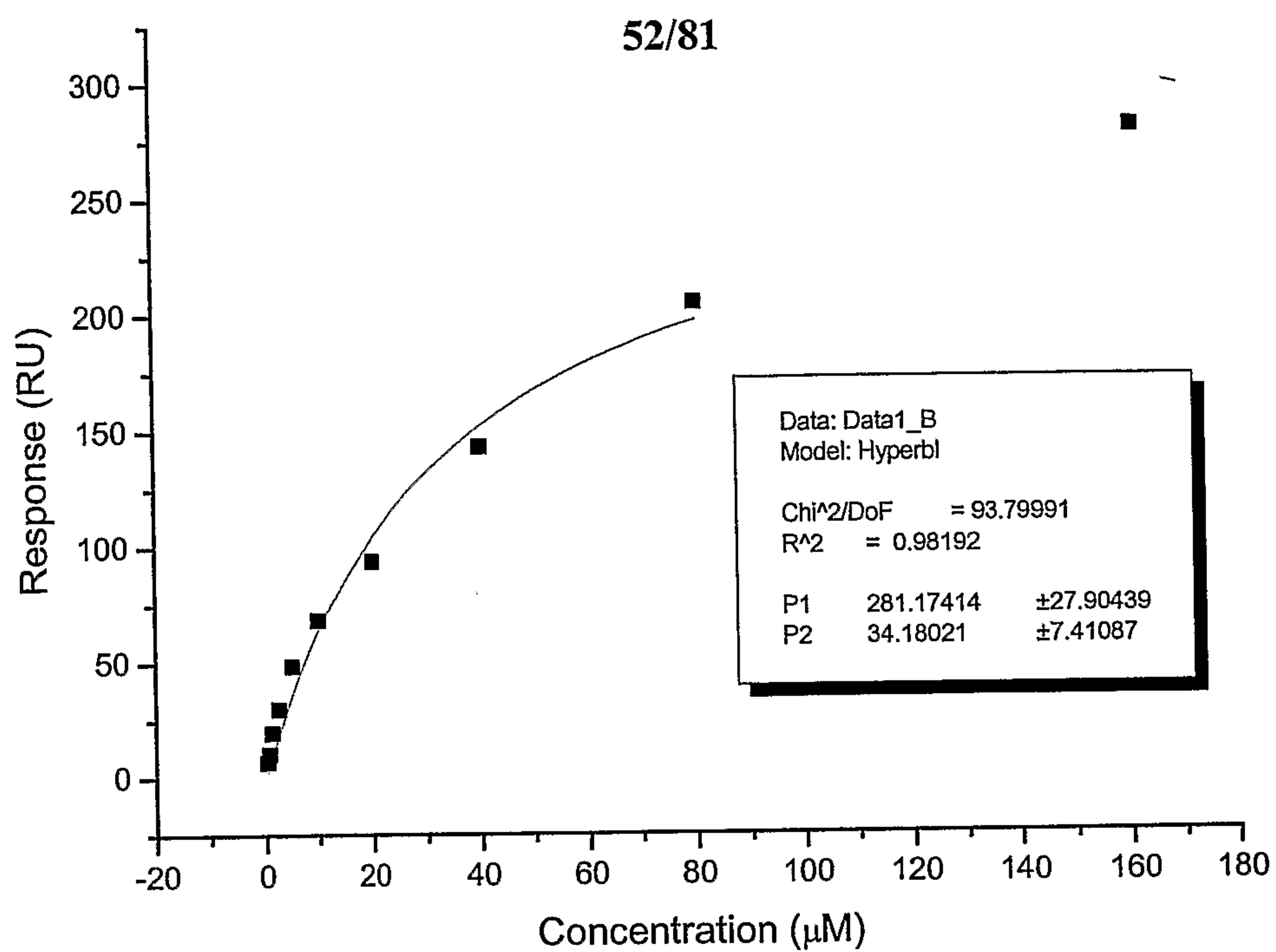
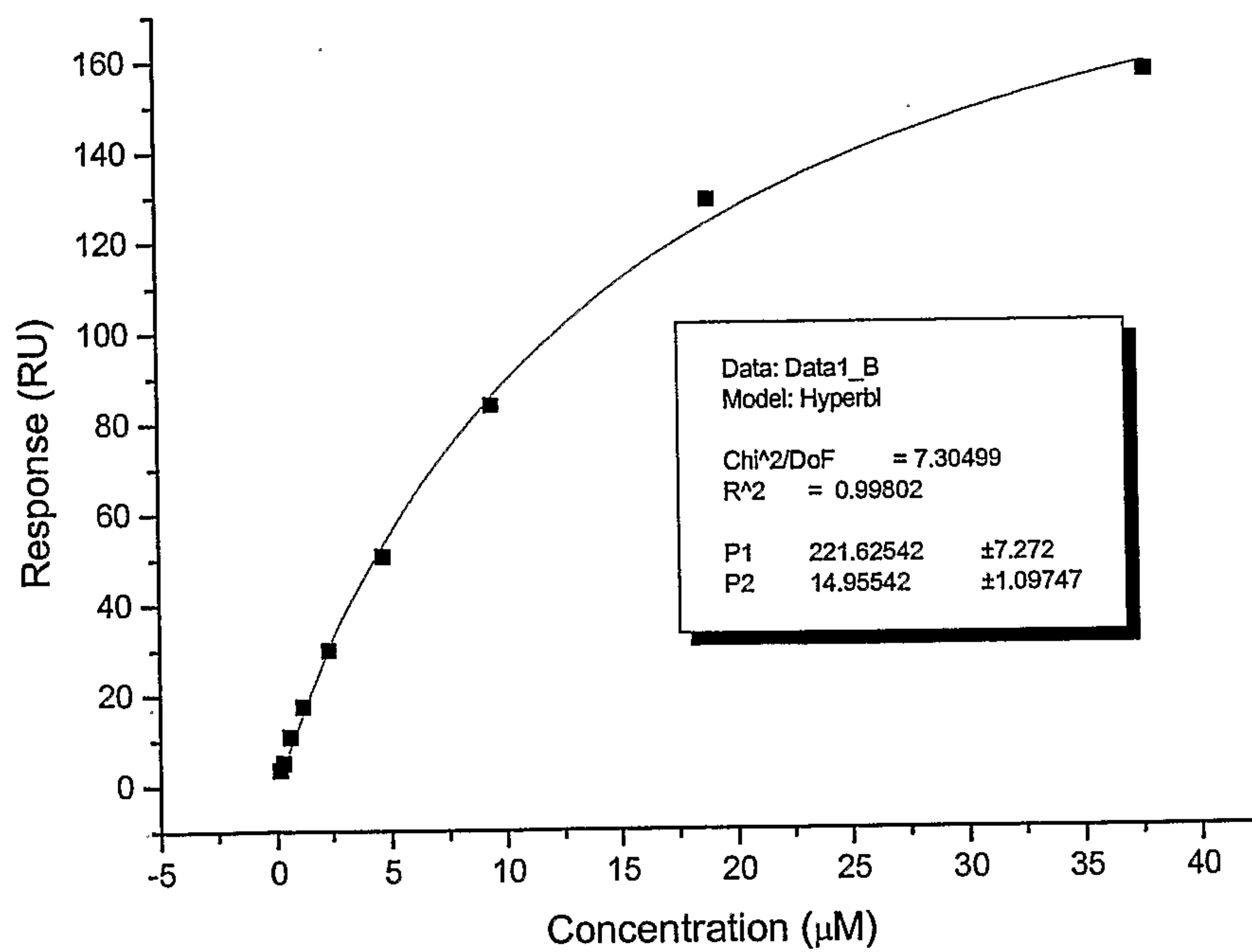


Figure 78: Thr 45/Ser 77

**Figure 79: Tyr 10/Ser 17****Figure 80: Thr 45/Asp 59**

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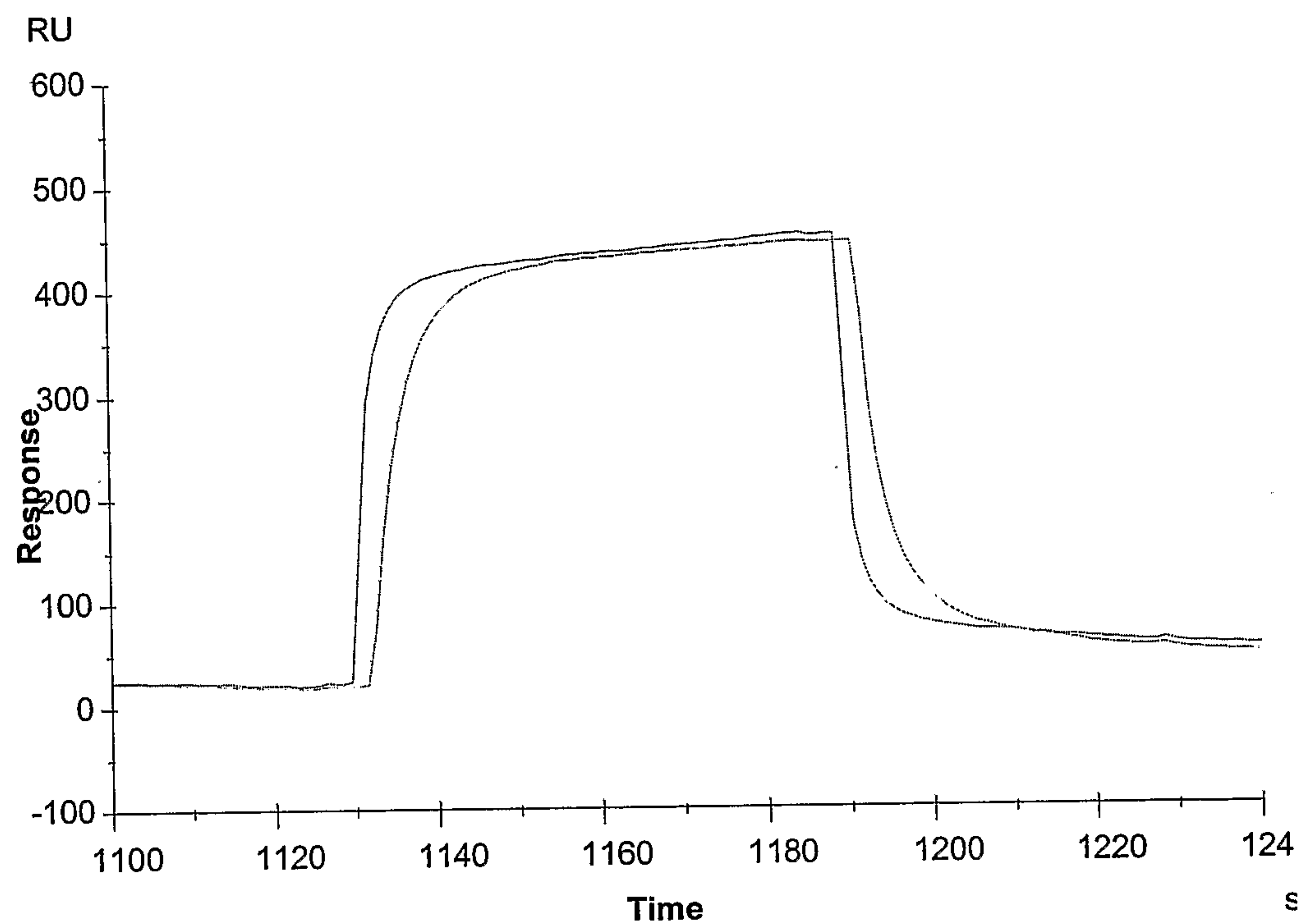


Figure 81: Met 52/Gly 55

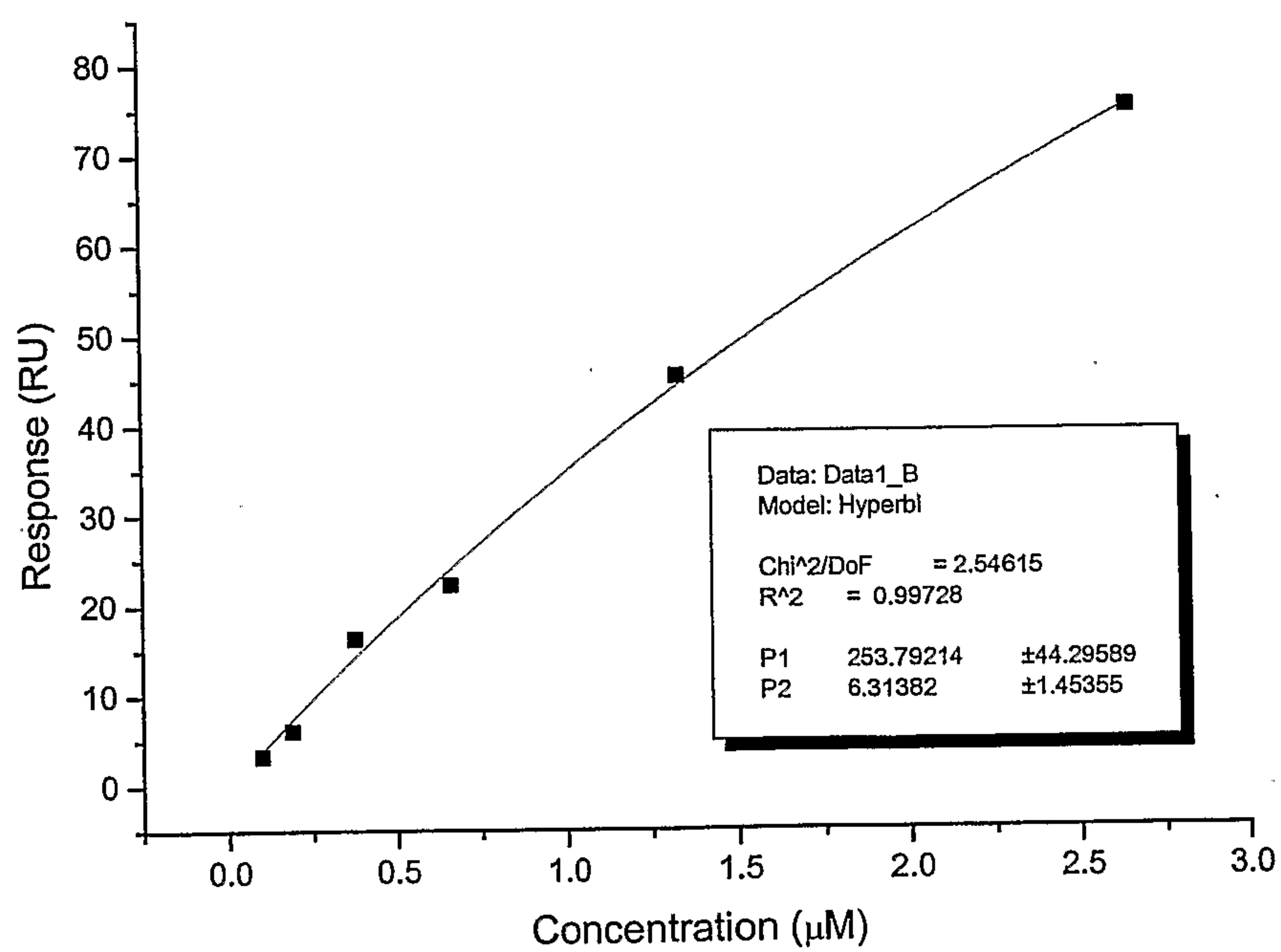


Figure 82: Ser 15/Glu 15

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Figure 83a

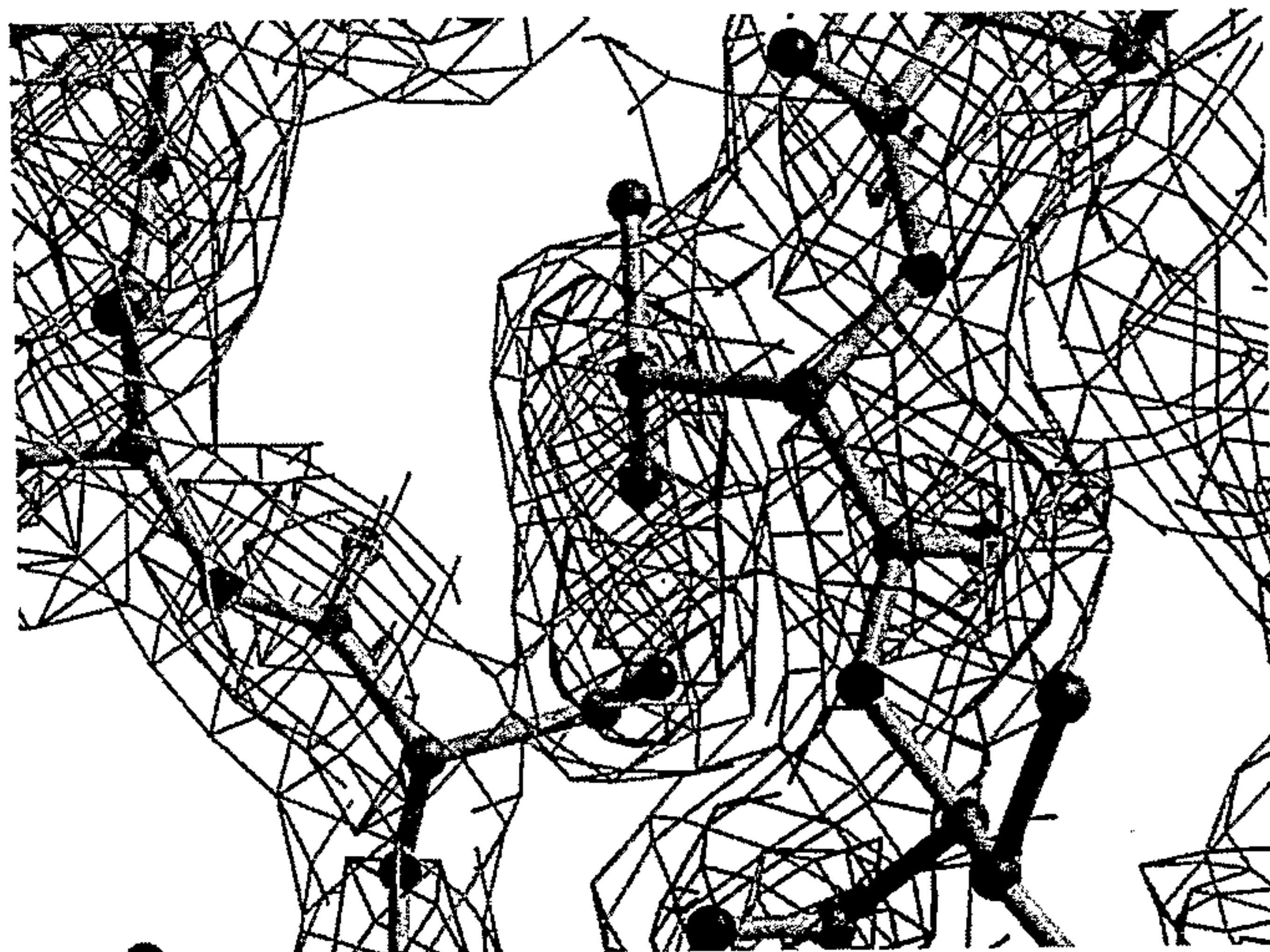
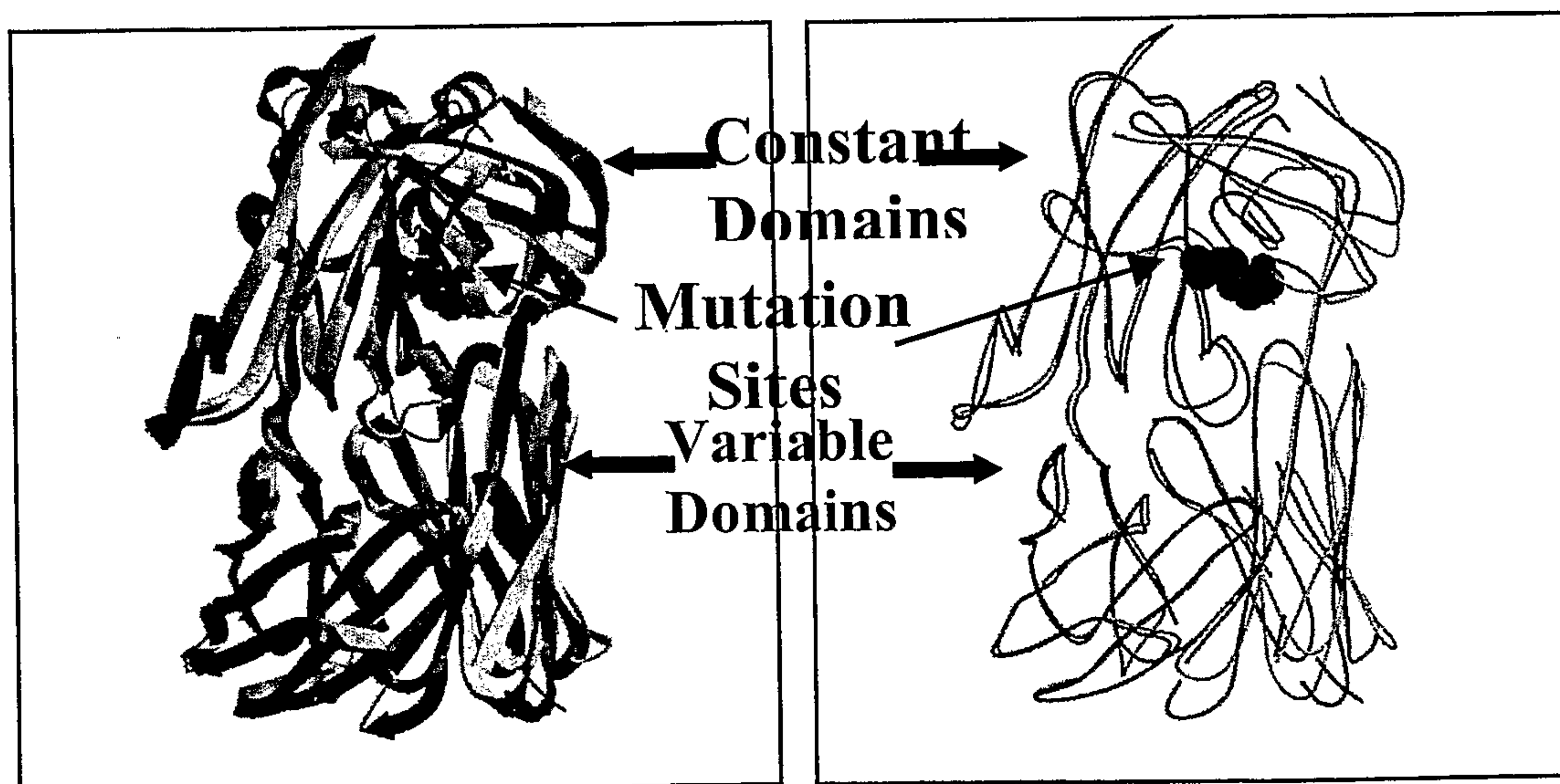


Figure 83b



Figure 84



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Figure 85a

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ctgaacgatatttttgaagctcagaaaatcgaatggcattaa

Figure 85b

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M
S W Y R Q D P G M G L R L I H Y S V G A G I T D Q G E V P N G
Y N V S R S T T E D F P L R L L S A A P S Q T S V Y F C A S S
Y V G N T G E L F F G E G S R L T V L E D L K N V F P P E V A
V F E P S E A E I S H T Q K A T L V C L A T G F Y P D H V E L
S W W V N G K E V H S G V C T D P Q P L K E Q P A L N D S R Y
A L S S R L R V S A T F W Q D P R N H F R C Q V Q F Y G L S E
N D E W T Q D R A K P V T Q I V S A E A W G R A D G S G G G L
N D I F E A Q K I E W H Stop

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Figure 86a

atgggtgtcactcagacccccaaaattccaggtcctgaagacaggacagagcatgaca
ctgcagtgtgcccaggatatgaaccatgaatacatgtcctggtatcgacaagaccca
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gtcaccagatcgtcagcgccgaggcctggggtagagcagacggatccggtggtggt
catcatcaccatcatcactaa

Figure 86b

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M
S W Y R Q D P G M G L R L I H Y S V G A G I T D Q G E V P N G
Y N V S R S T T E D F P L R L L S A A P S Q T S V Y F C A S S
Y V G N T G E L F F G E G S R L T V L E D L K N V F P P E V A
V F E P S E A E I S H T Q K A T L V C L A T G F Y P D H V E L
S W W V N G K E V H S G V C T D P Q P L K E Q P A L N D S R Y
A L S S R L R V S A T F W Q D P R N H F R C Q V Q F Y G L S E
N D E W T Q D R A K P V T Q I V S A E A W G R A D G S G G G H
H H H H H Stop

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Figure 87

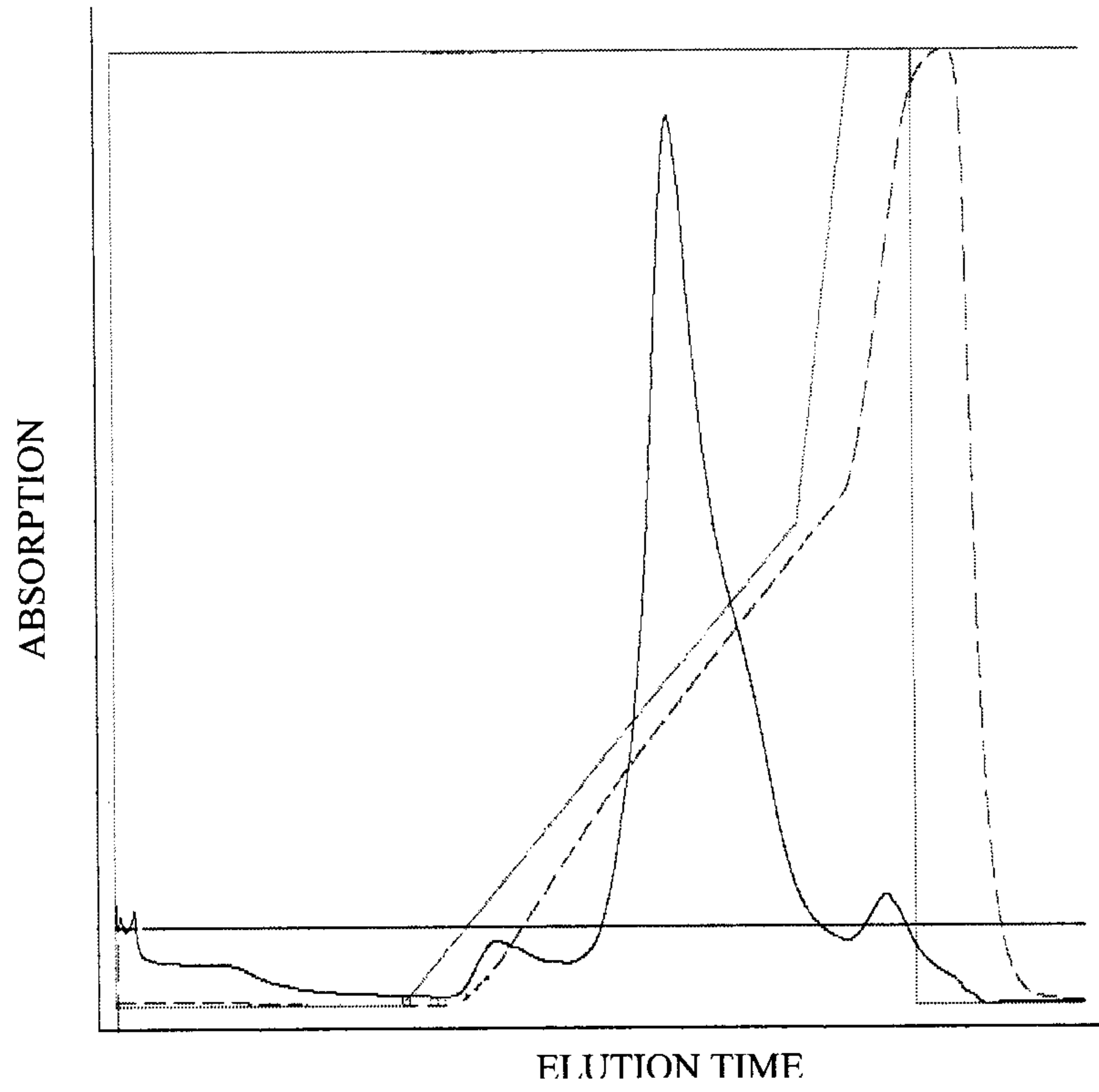
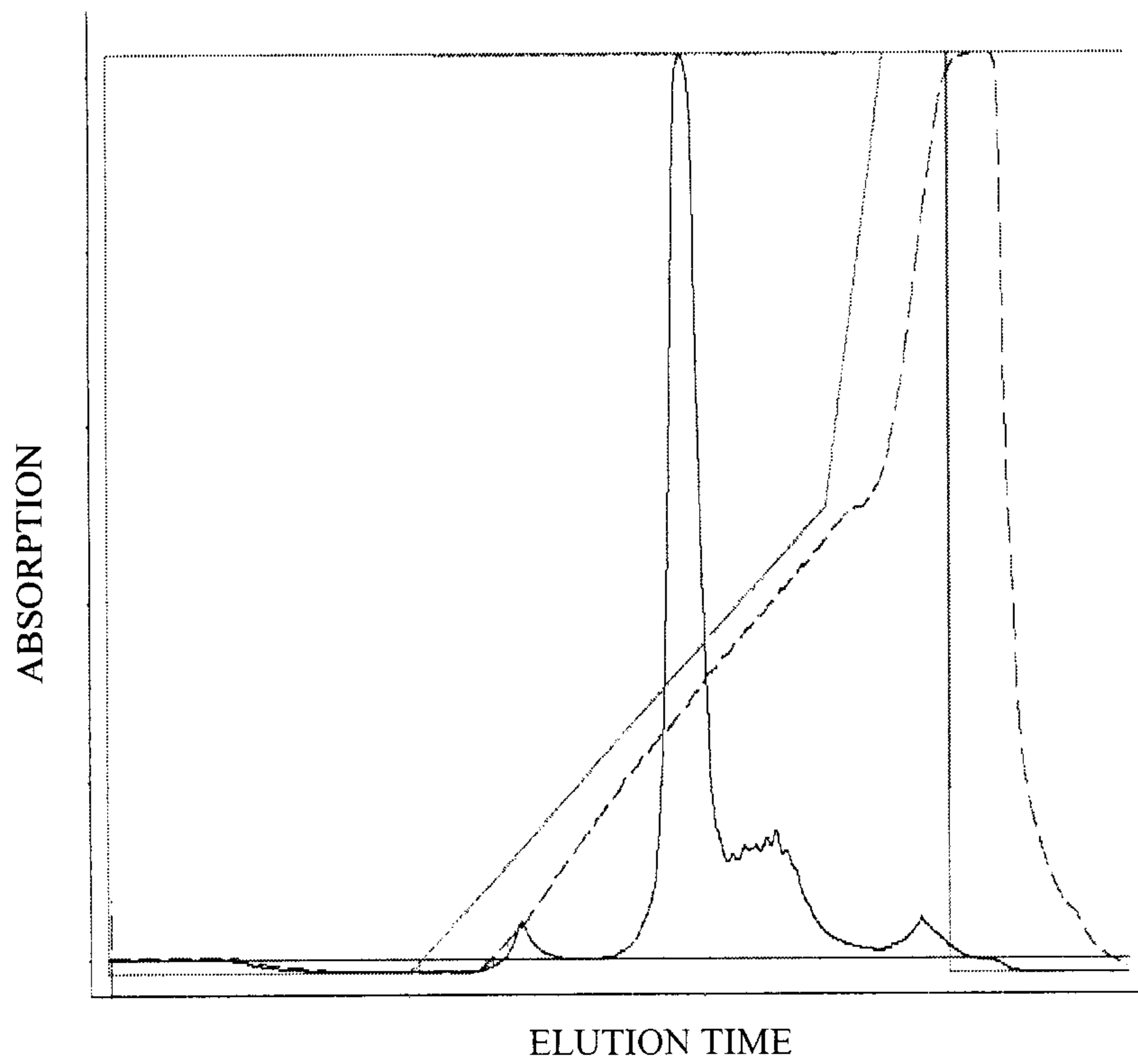


Figure 88



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Figure 89

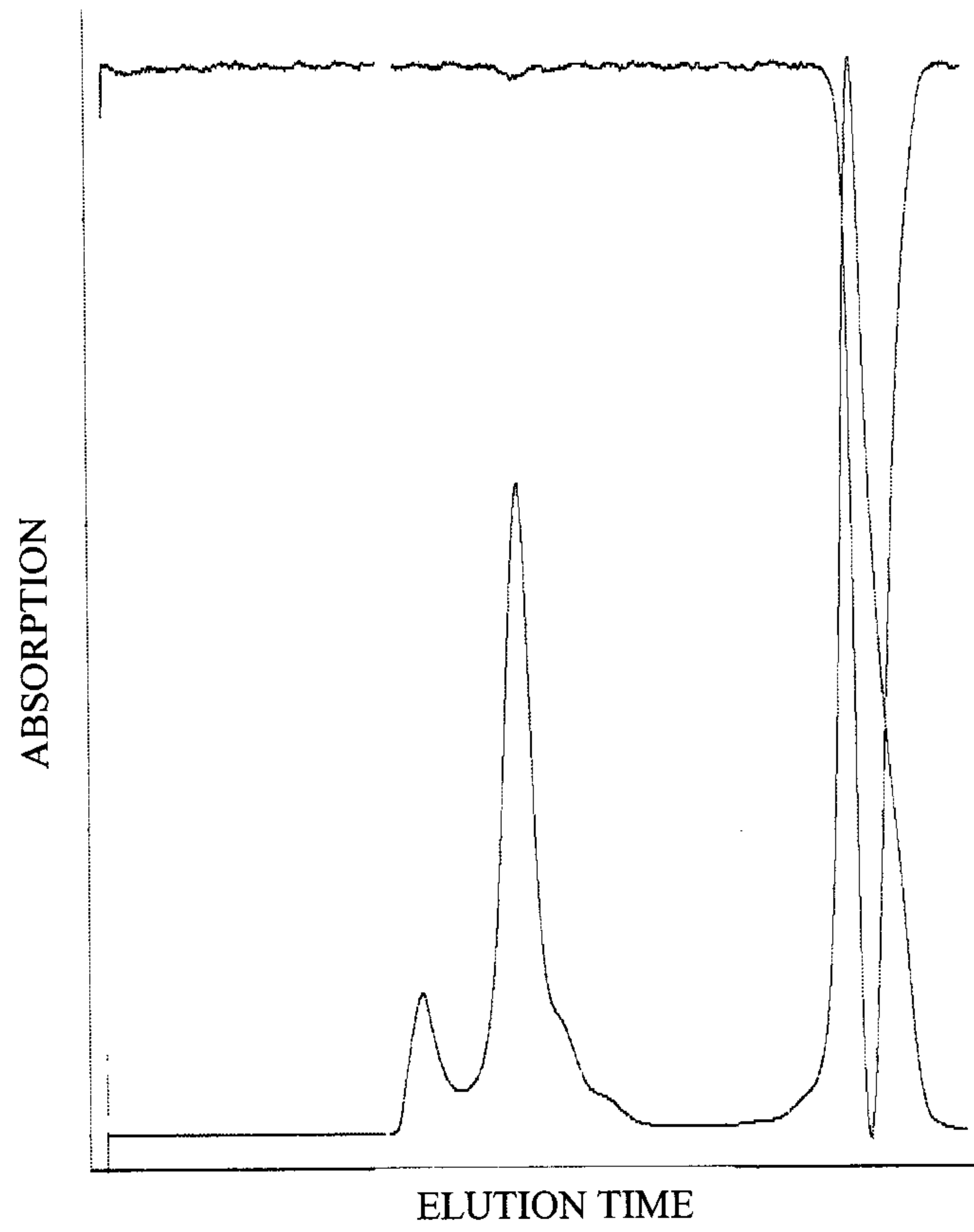
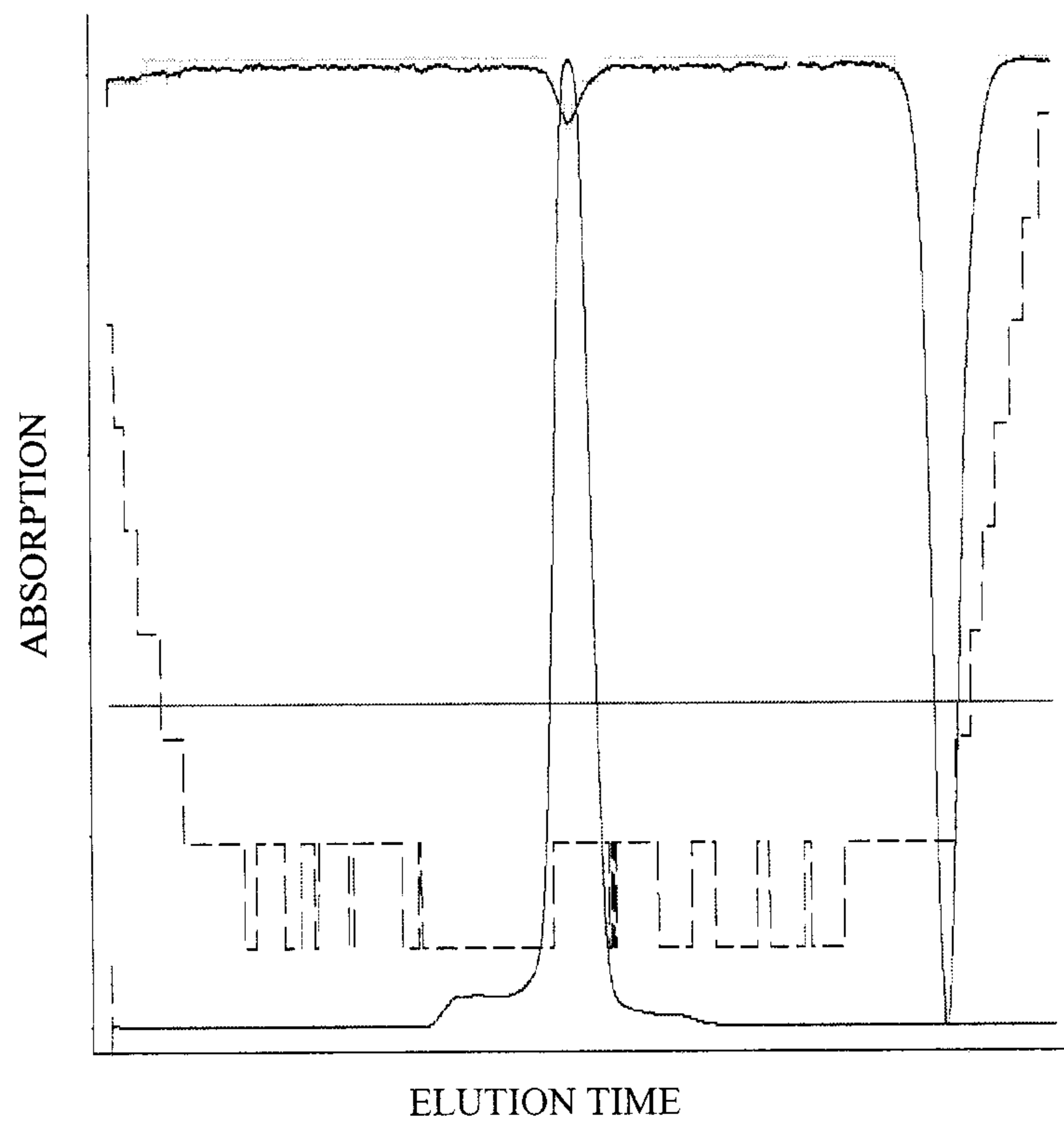
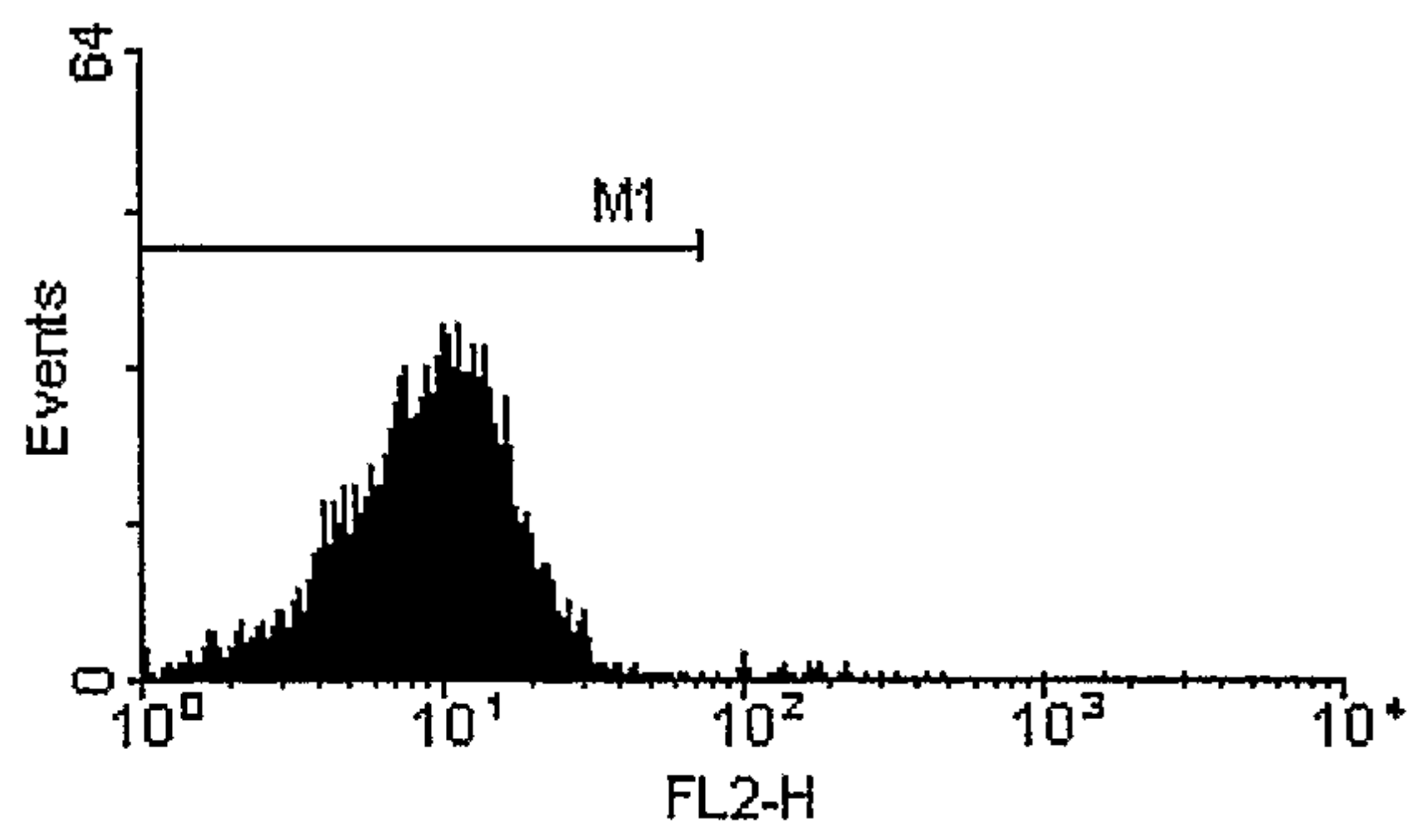
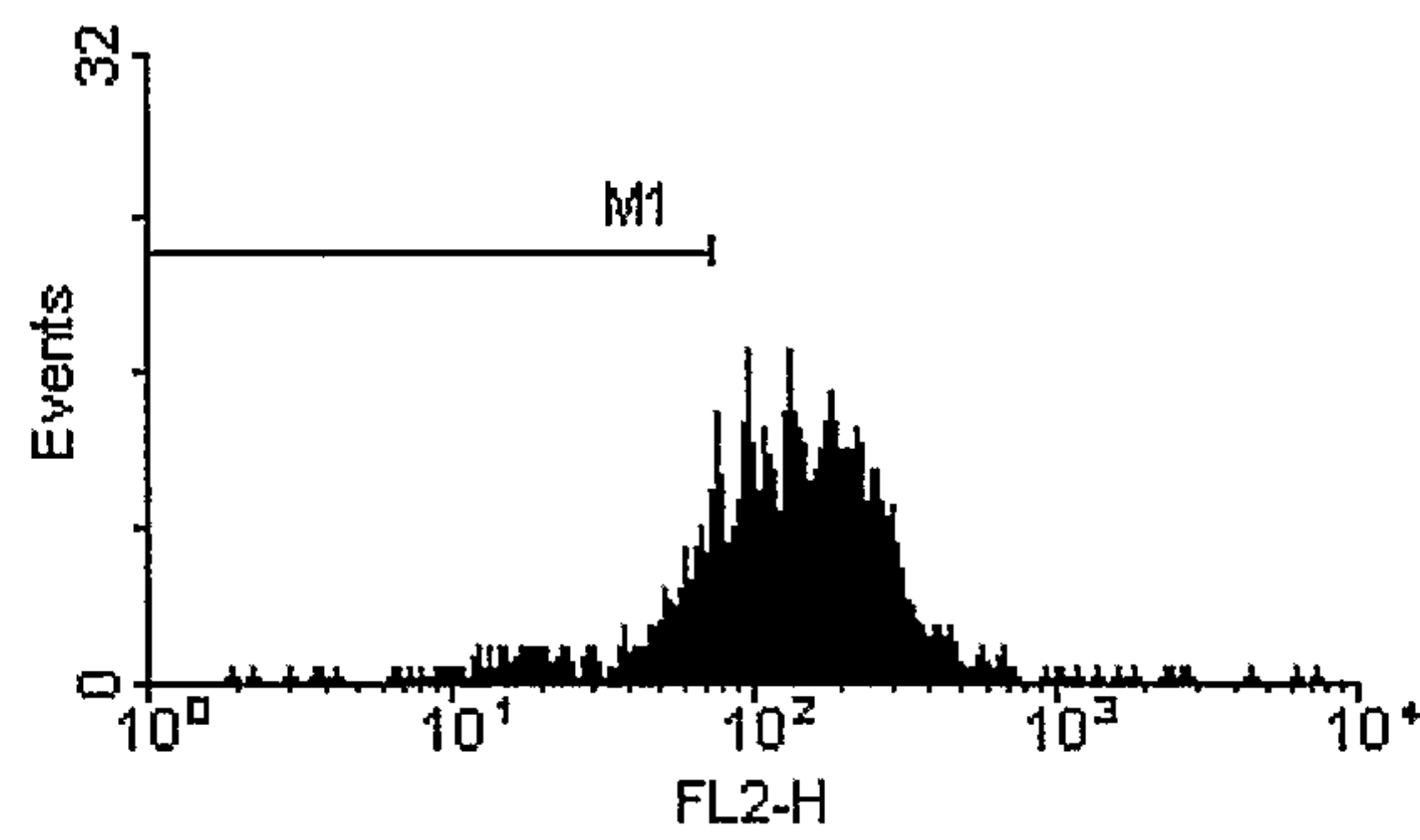
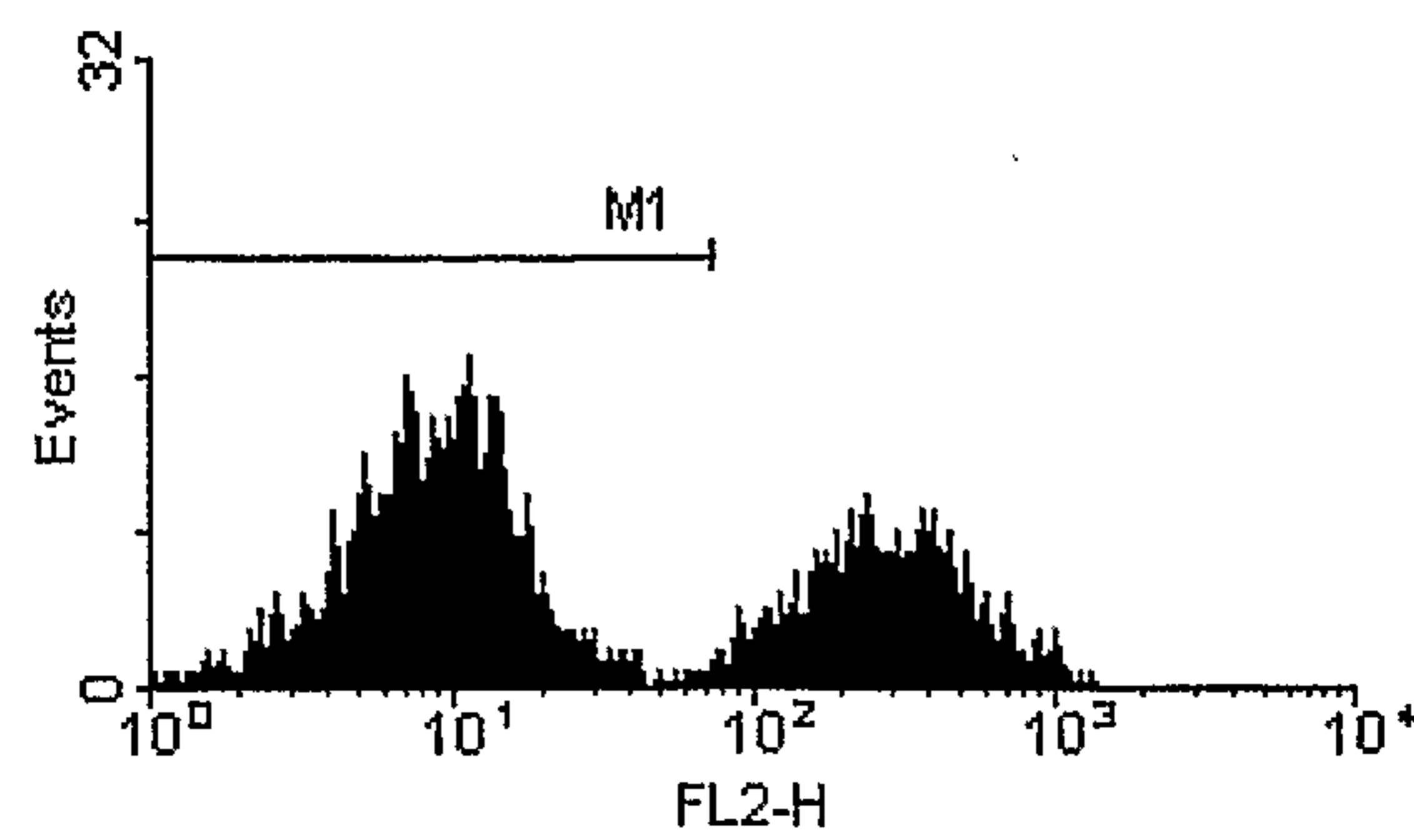


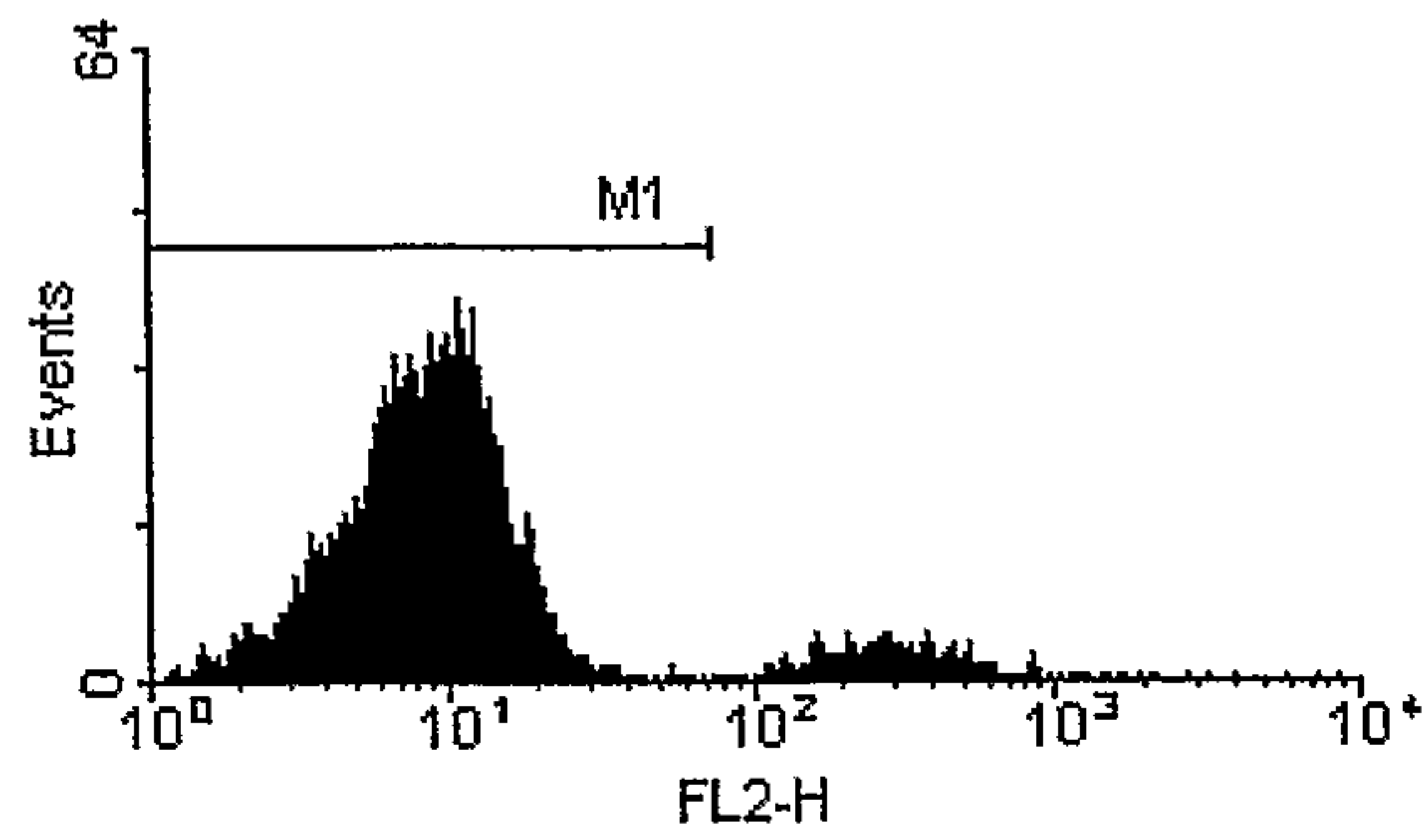
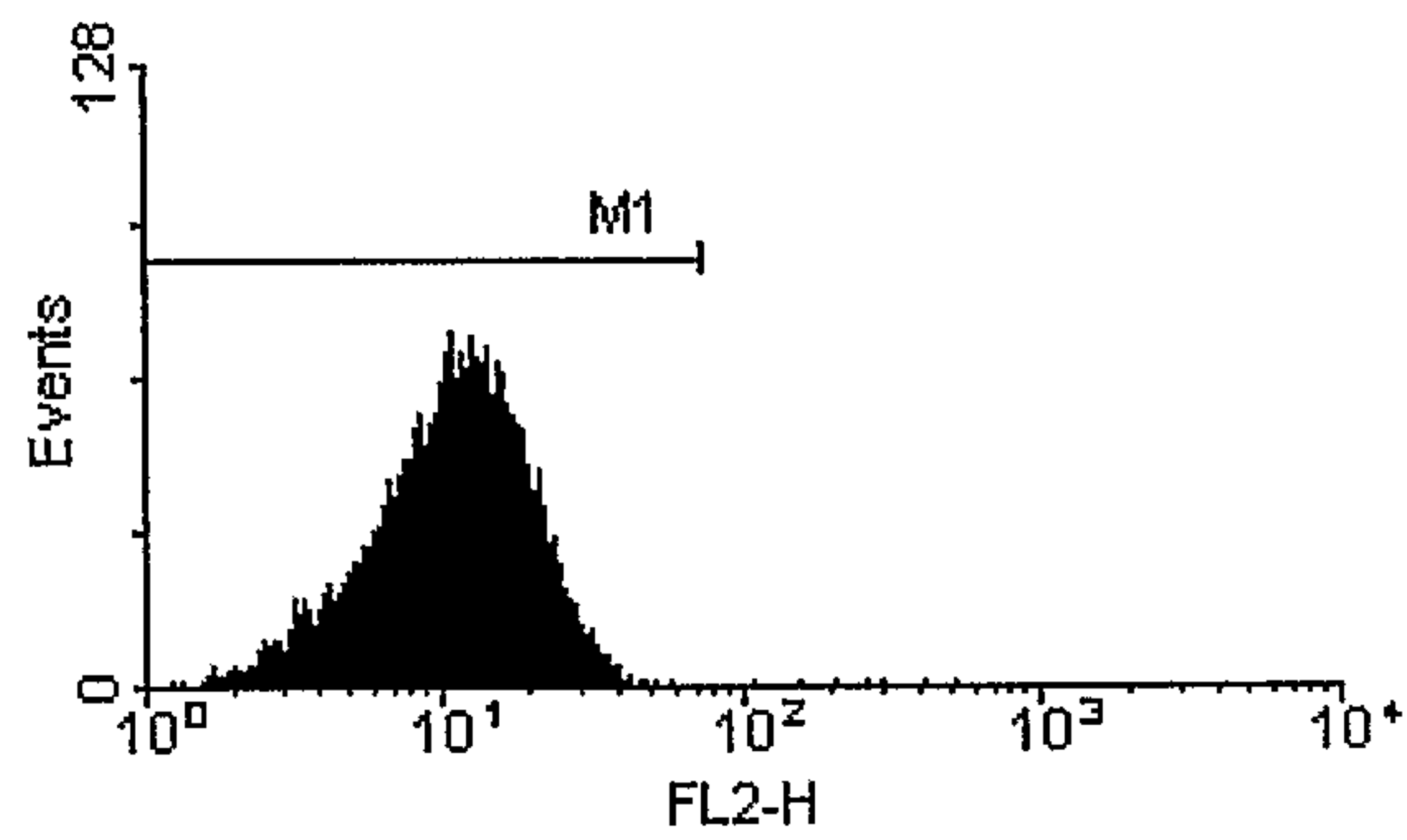
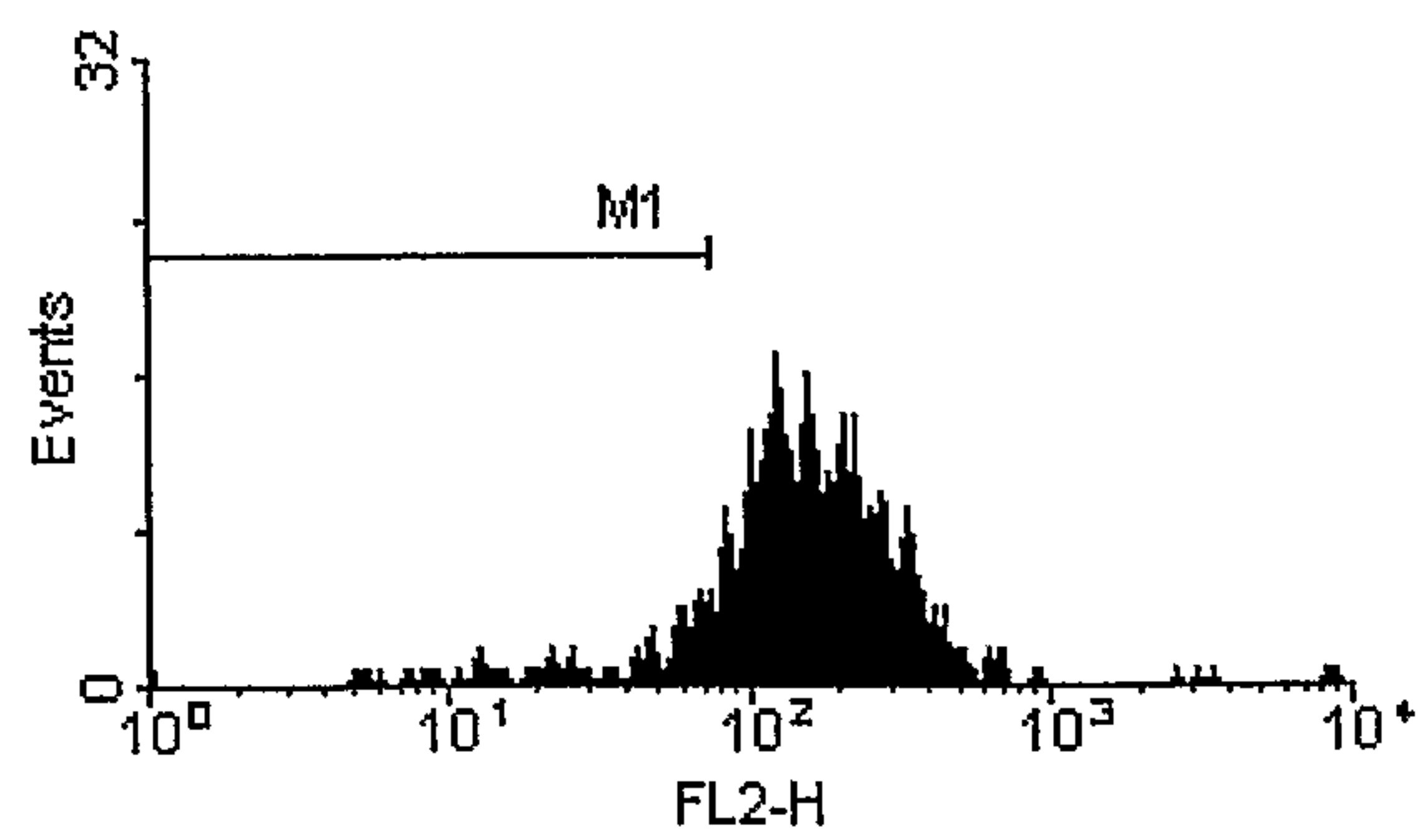
Figure 90



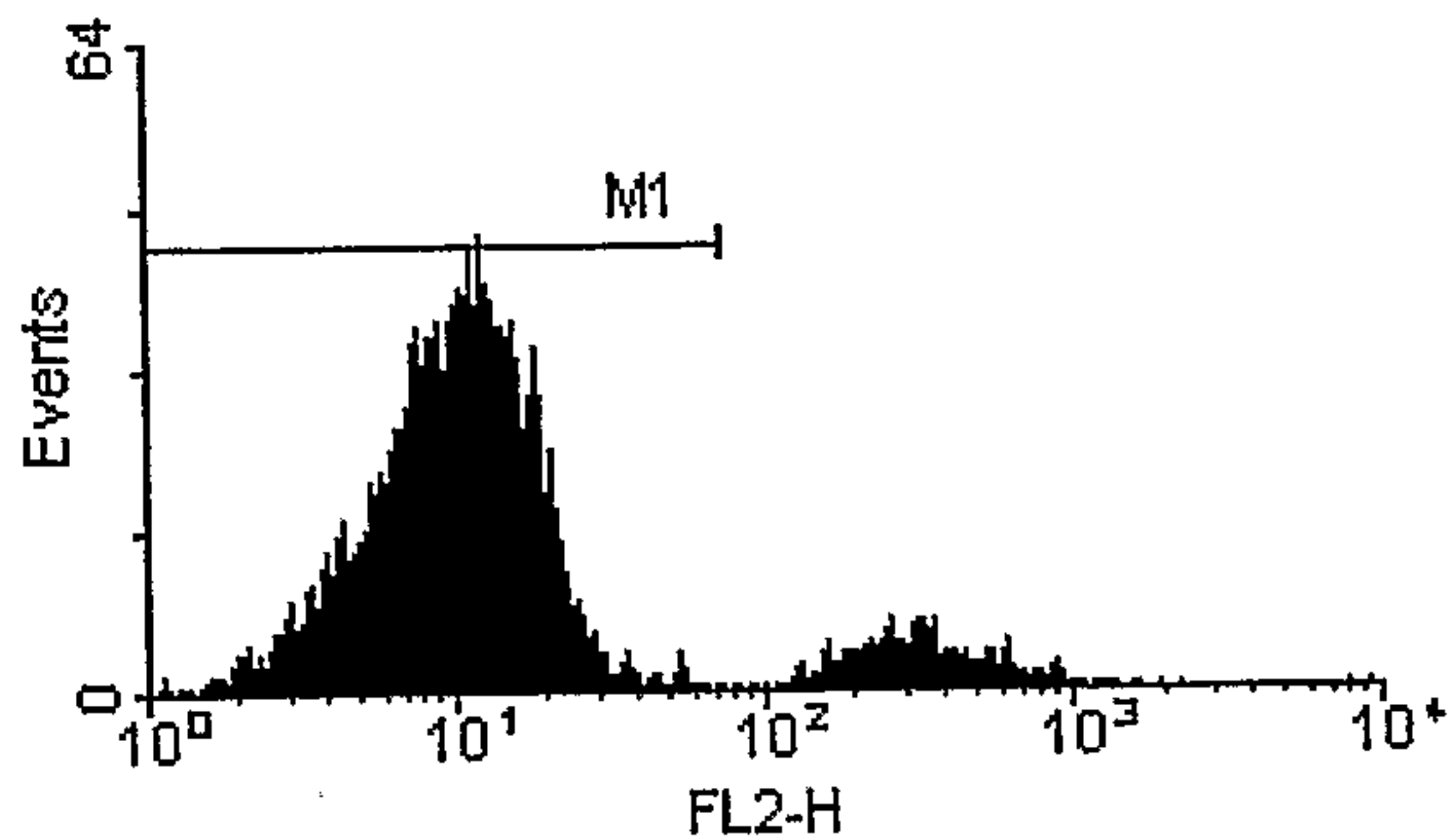
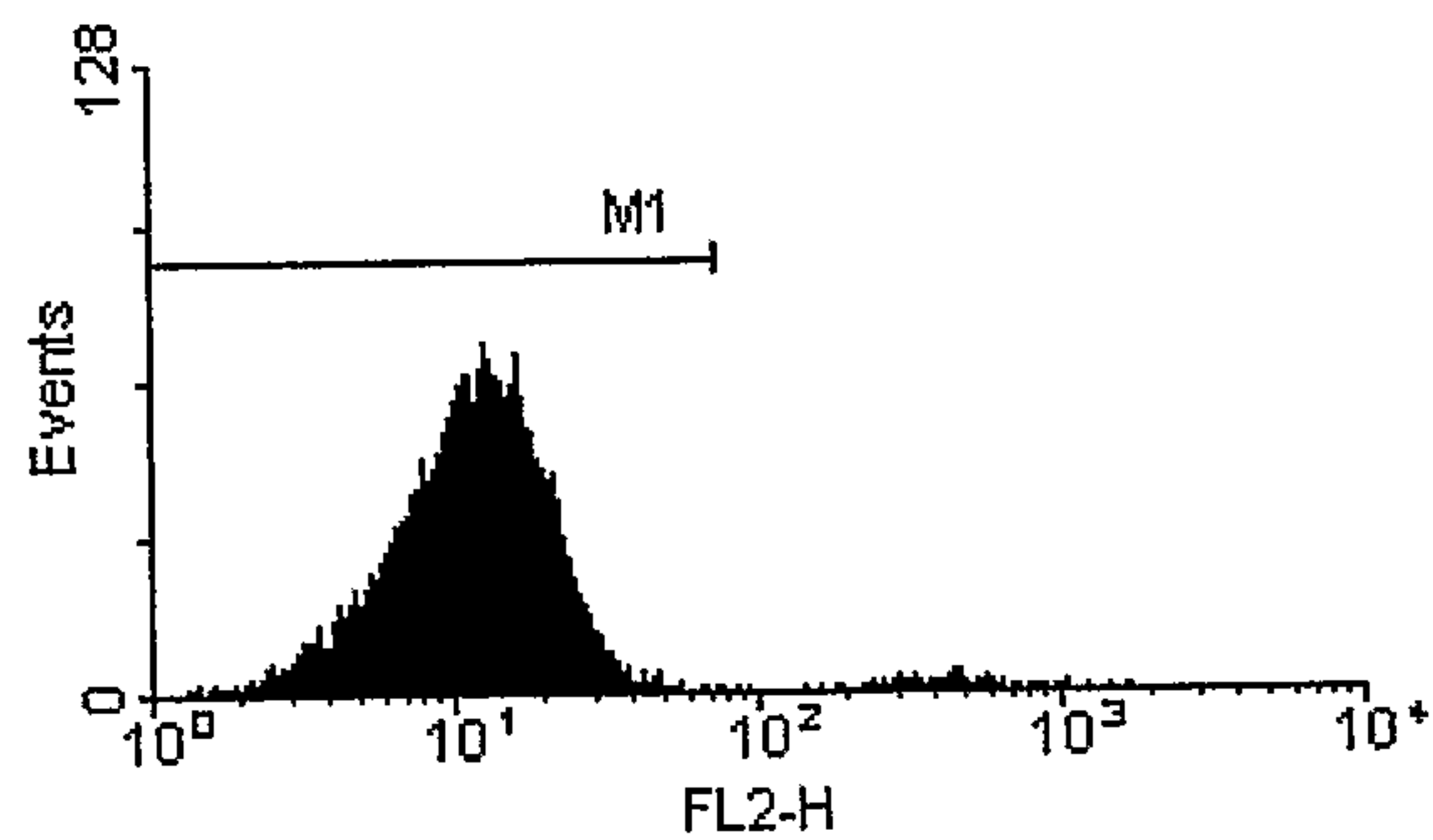
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**Figure 91a. PP LCL NYESO 0 TCR $5\mu\text{g}$** **Figure 91b. PP LCL NYESO 10^{-4}M TCR $5\mu\text{g}$** **Figure 91c. PP LCL NYESO 10^{-5}M TCR $5\mu\text{g}$**

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**Figure 91d. PP LCL NYESO 10^{-6} M TCR $5\mu\text{g}$** **Figure 91e. PP LCL NYESO 0 TCR $10\mu\text{g}$** **Figure 91f. PP LCL NYESO 10^{-4} M TCR $10\mu\text{g}$**

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Figure 91g. PP LCL NYESO 10^{-5} M TCR $10\mu\text{g}$ Figure 91h. PP LCL NYESO 10^{-6} M TCR $10\mu\text{g}$

atgaacgctggtgtcactcagacccccaaaattccaggtcctgaagacaggacagagc
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gaccagggcatggggctgaggctgattcattactcagttggtgctggtatcactgac
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tctctgagcagccgcctgagggtctcggccaccttctggcaggacccccgcaaccac
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Figure 92

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Figure 93

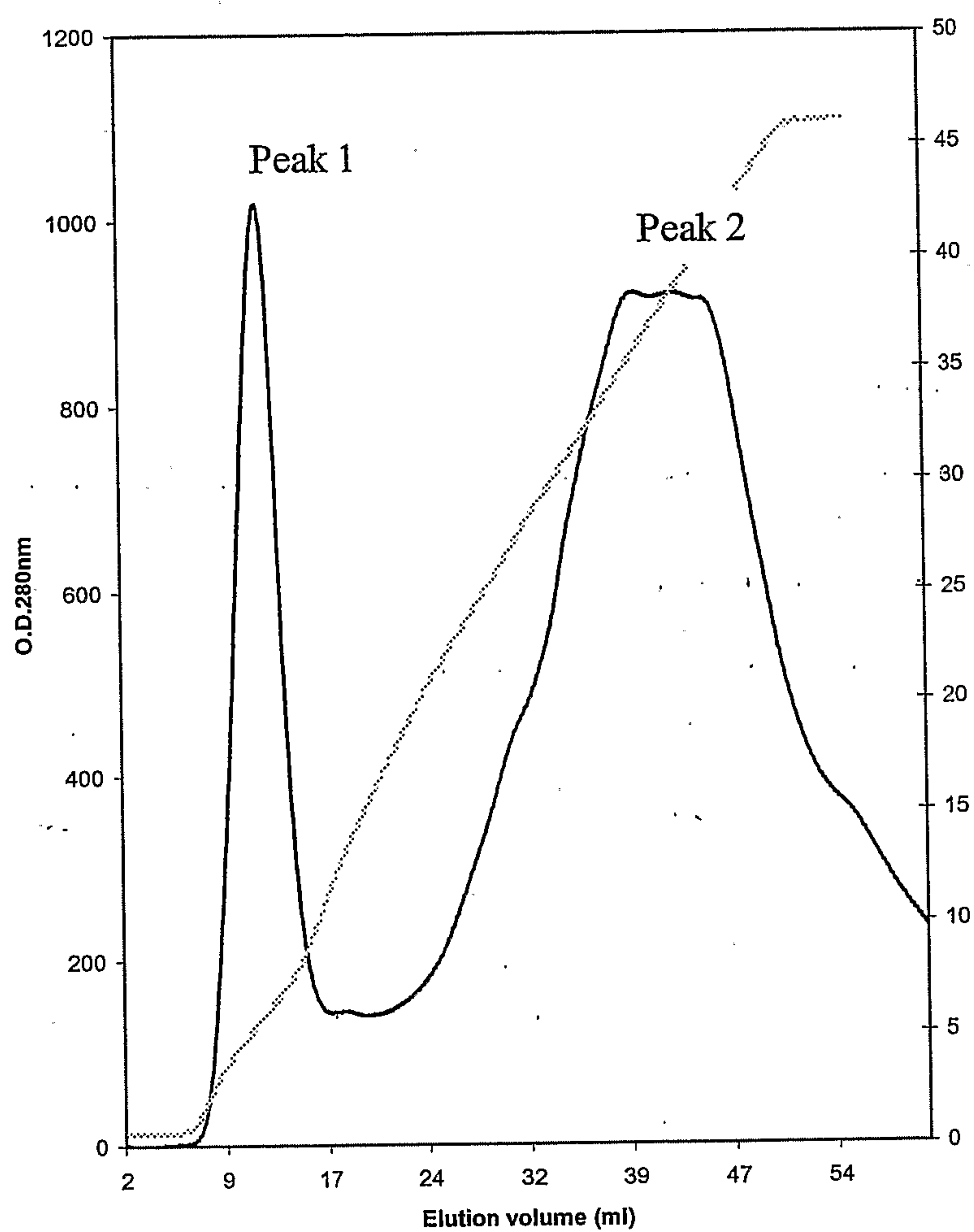
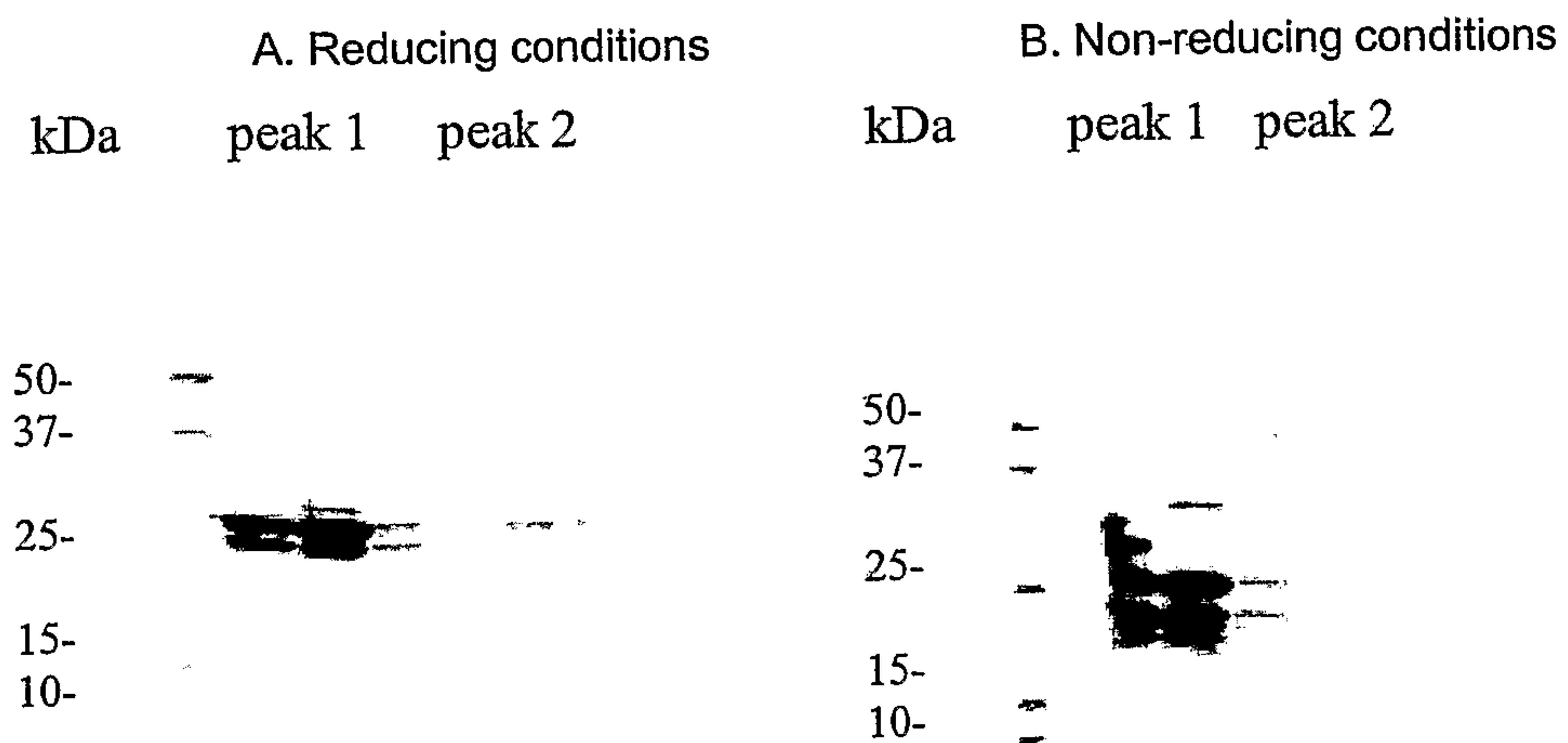
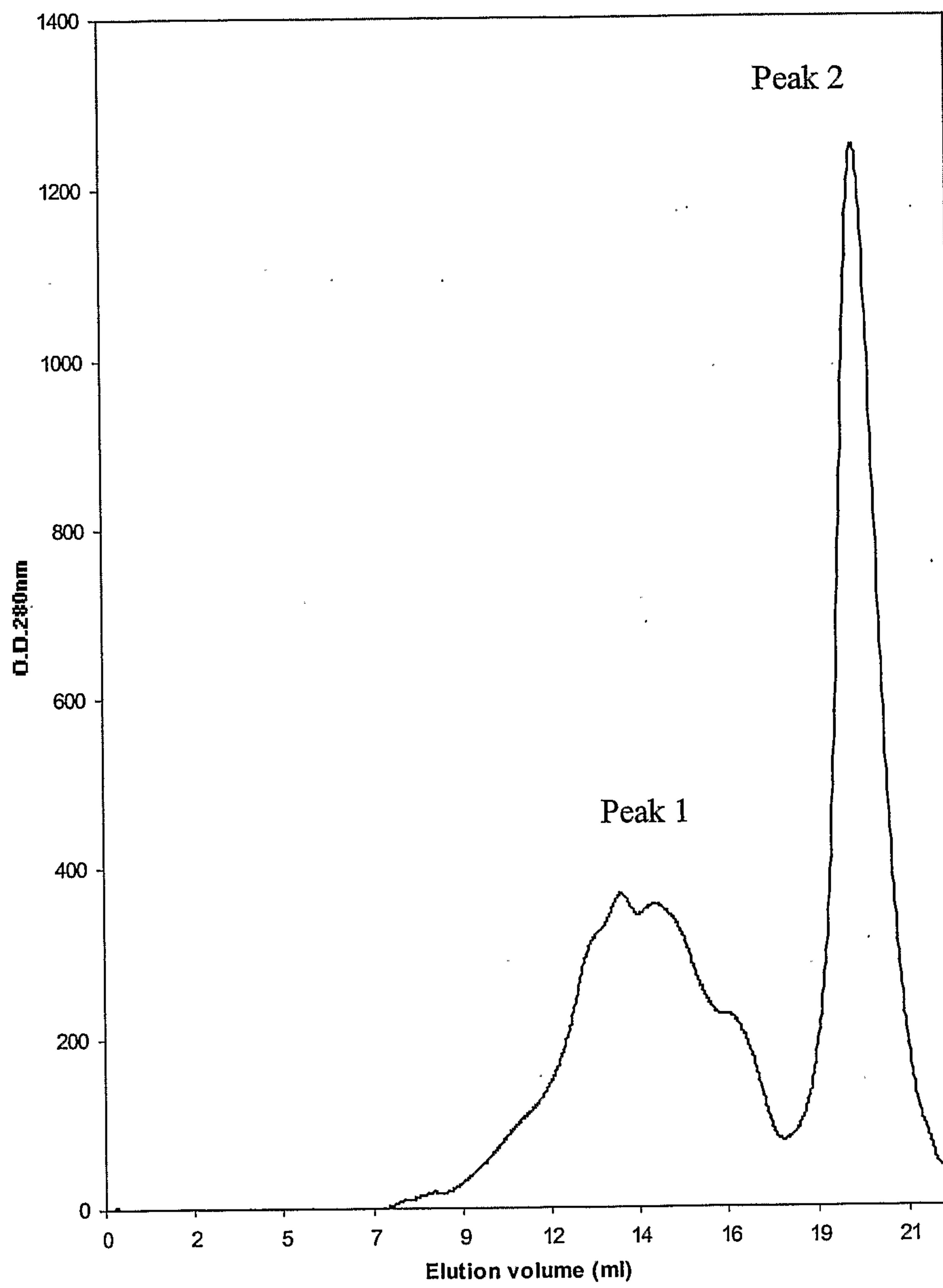


Figure 94



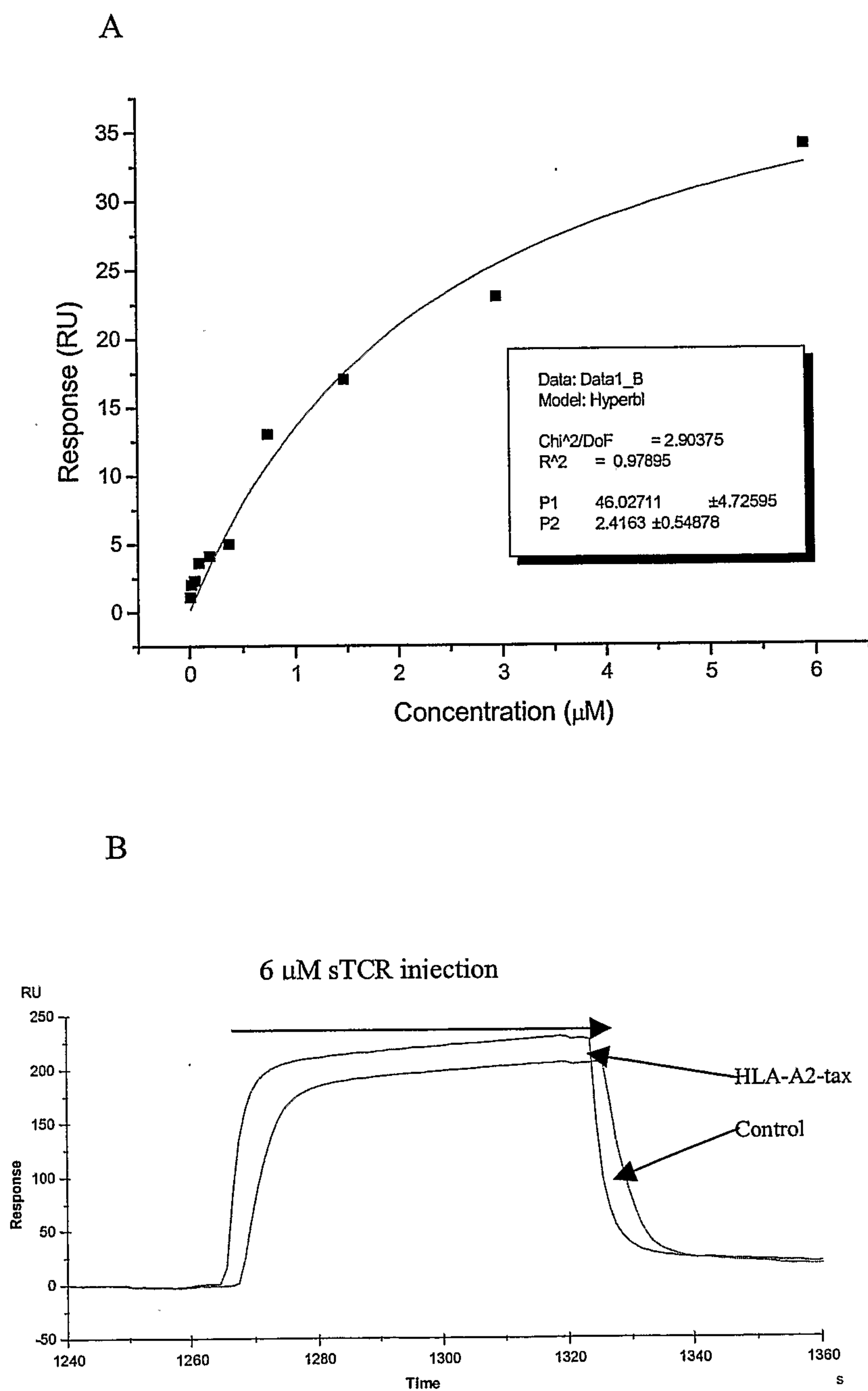
63/81

Figure 95



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Figure 96



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Figure 97

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gtcacagaggacctgaaaaacgtgttcccacccgagggtcgctgtgtttgagccatca
gaagcagagatctcccacacccaaaaggccacactgggtgtgcctggccacagggttc
taccgacacacgtggagctgagctgggtgggtgaatgggaaggaggtgcacagtggg
gtctgcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatac
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Figure 98

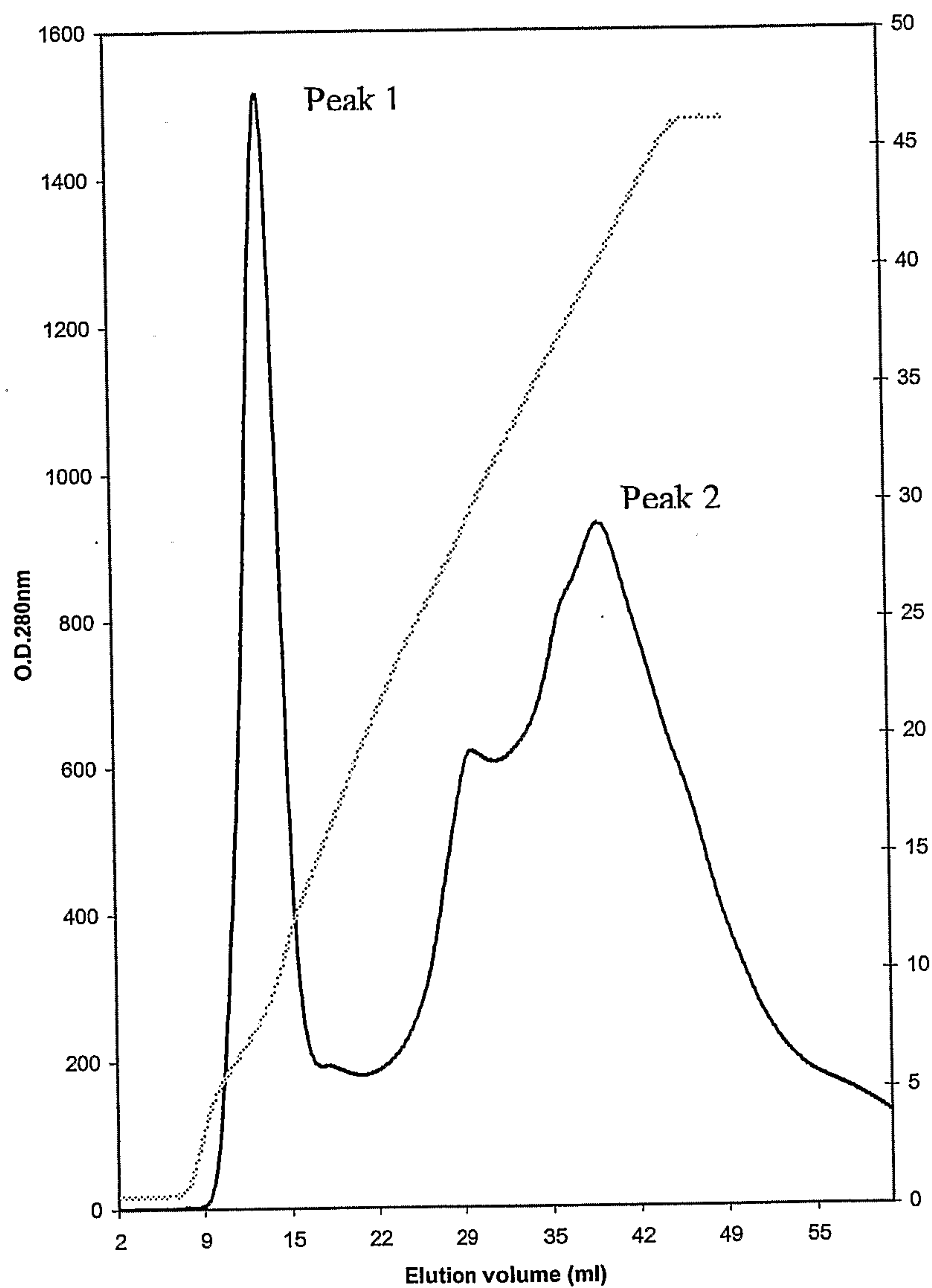
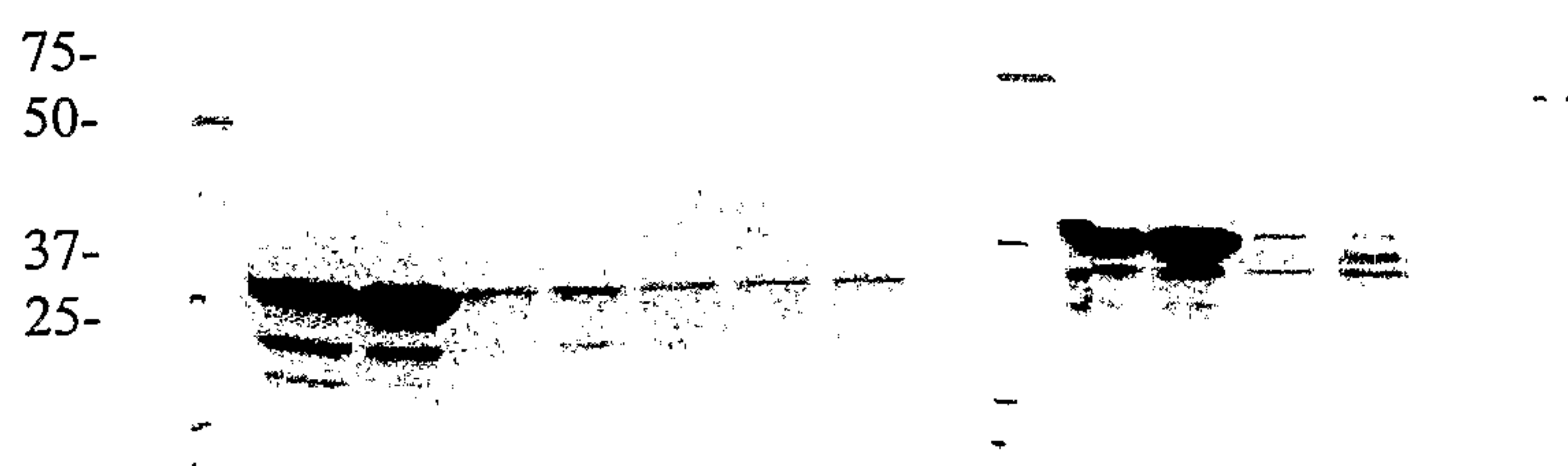


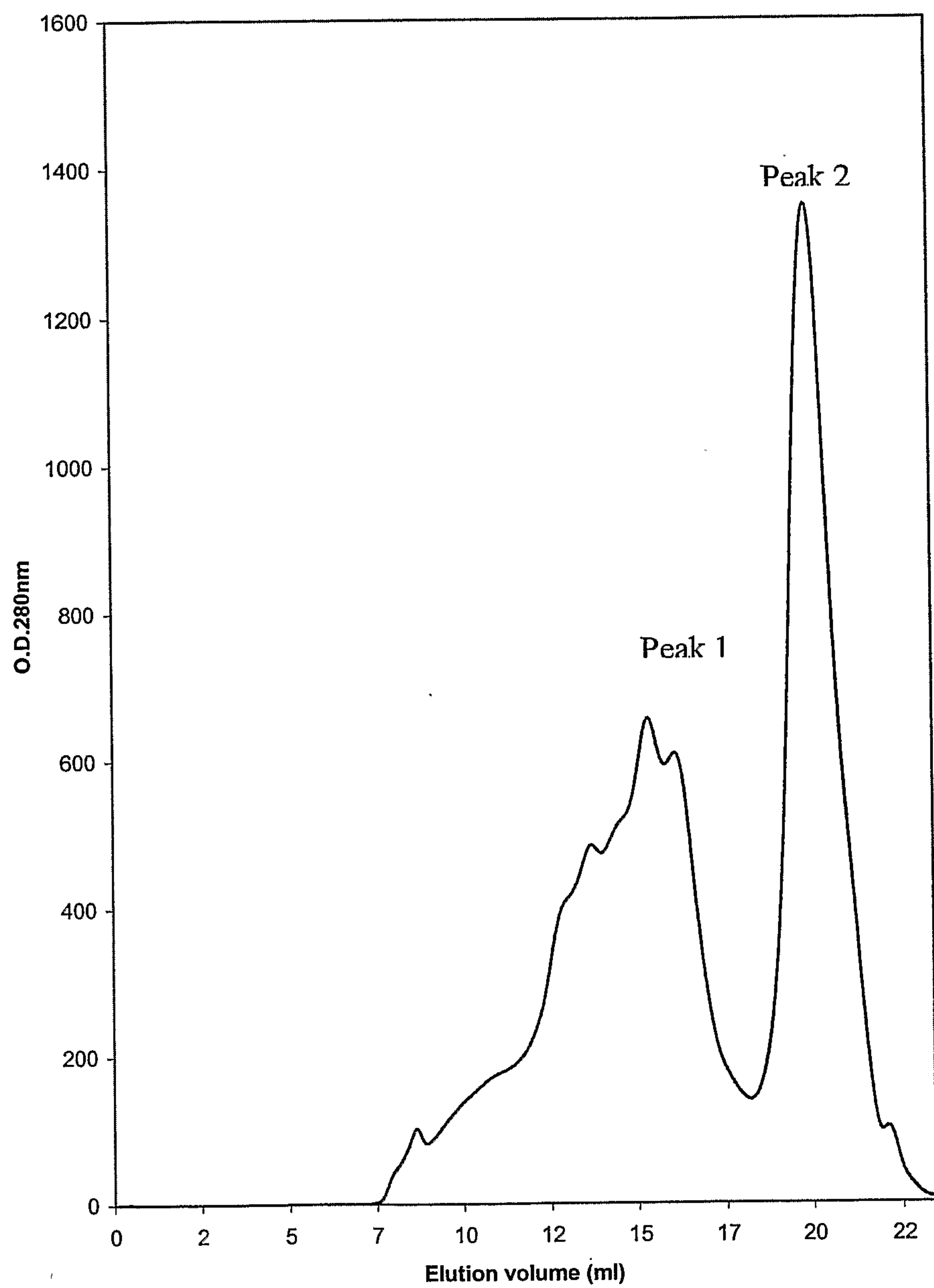
Figure 99

A. Reducing conditions B. Non-reducing conditions

KDa peak 1 peak 2 peak 1 peak 2



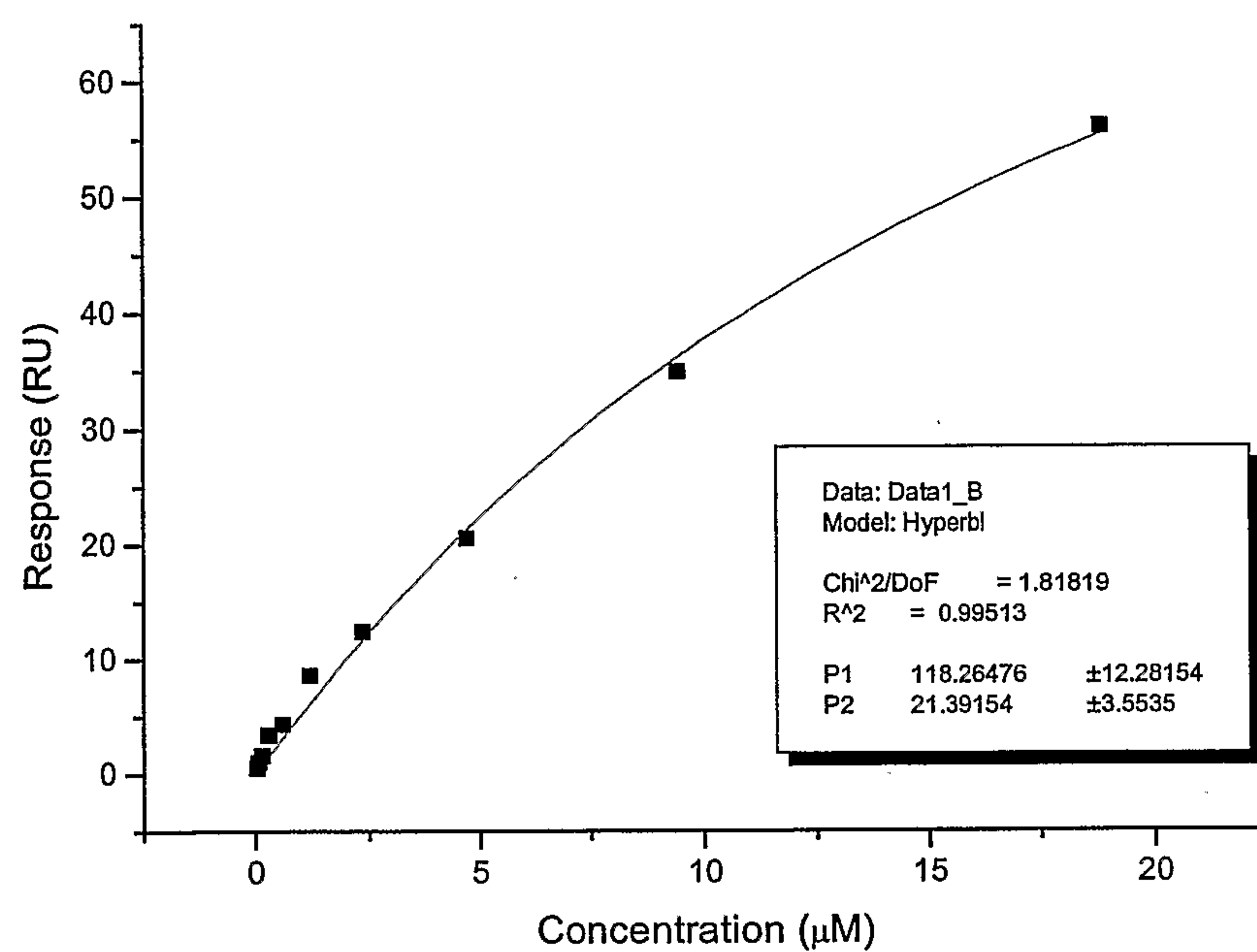
67/81
Figure 100



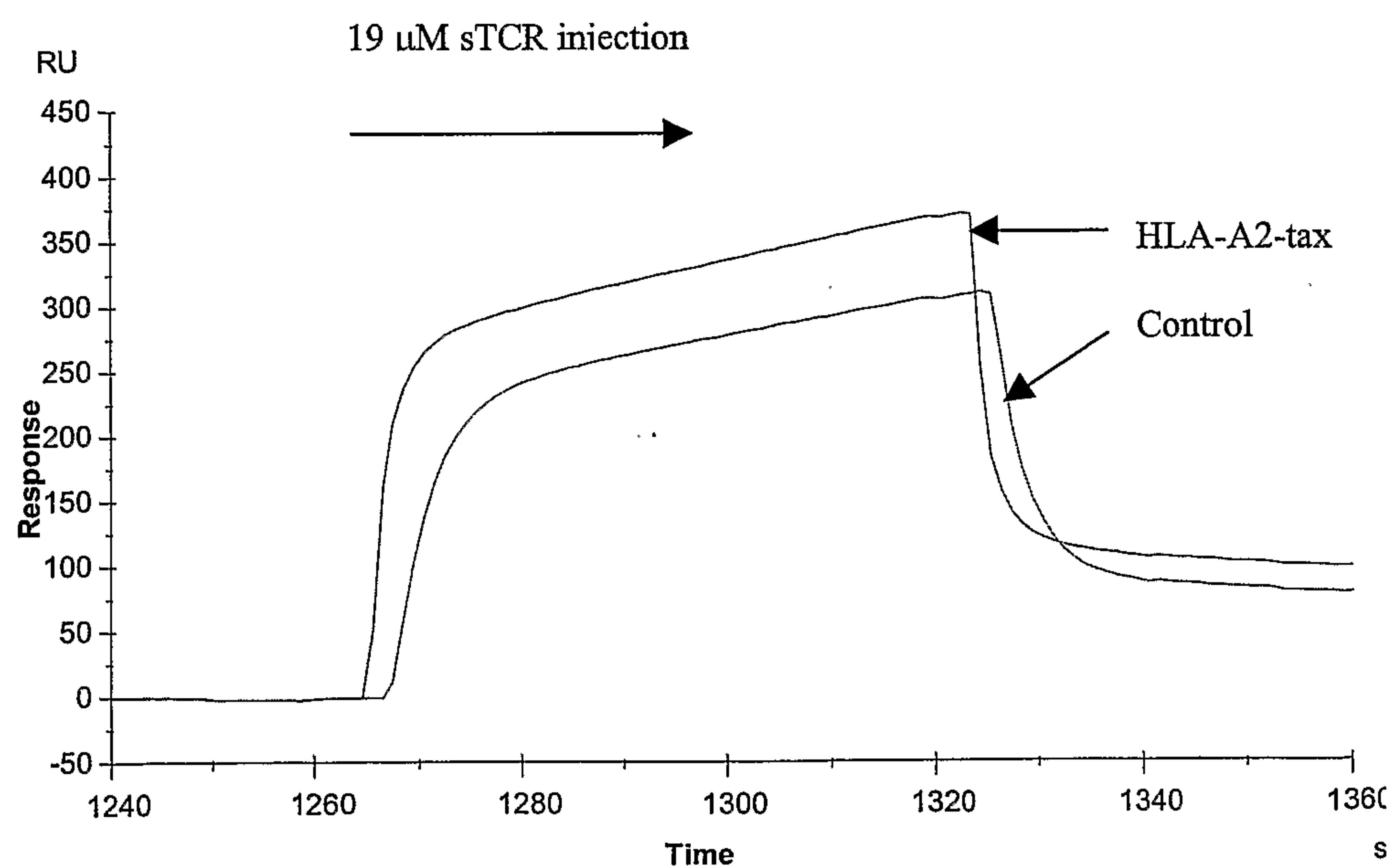
68/81

Figure 101

A



B



69/81

Figure 102

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70/81
Figure 103

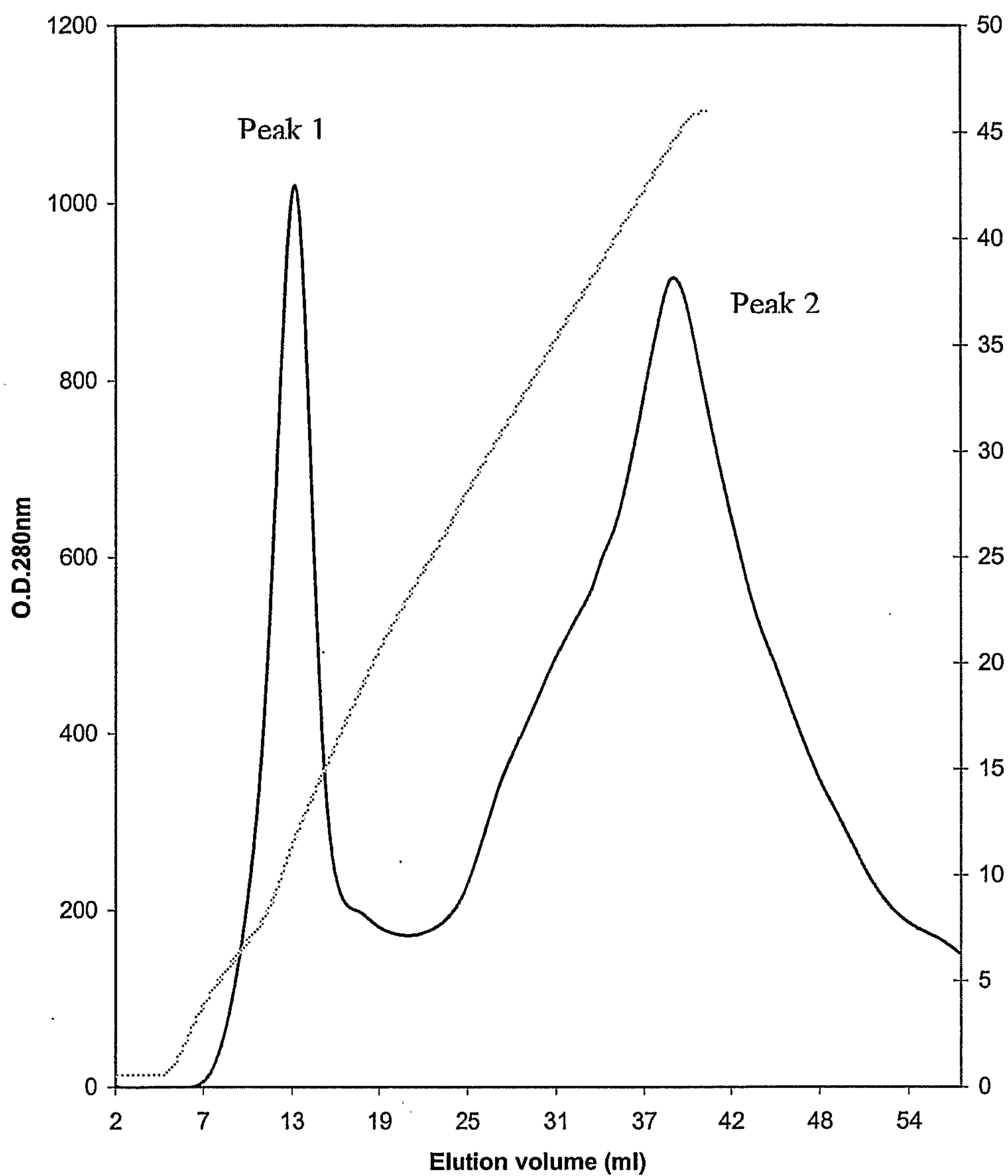
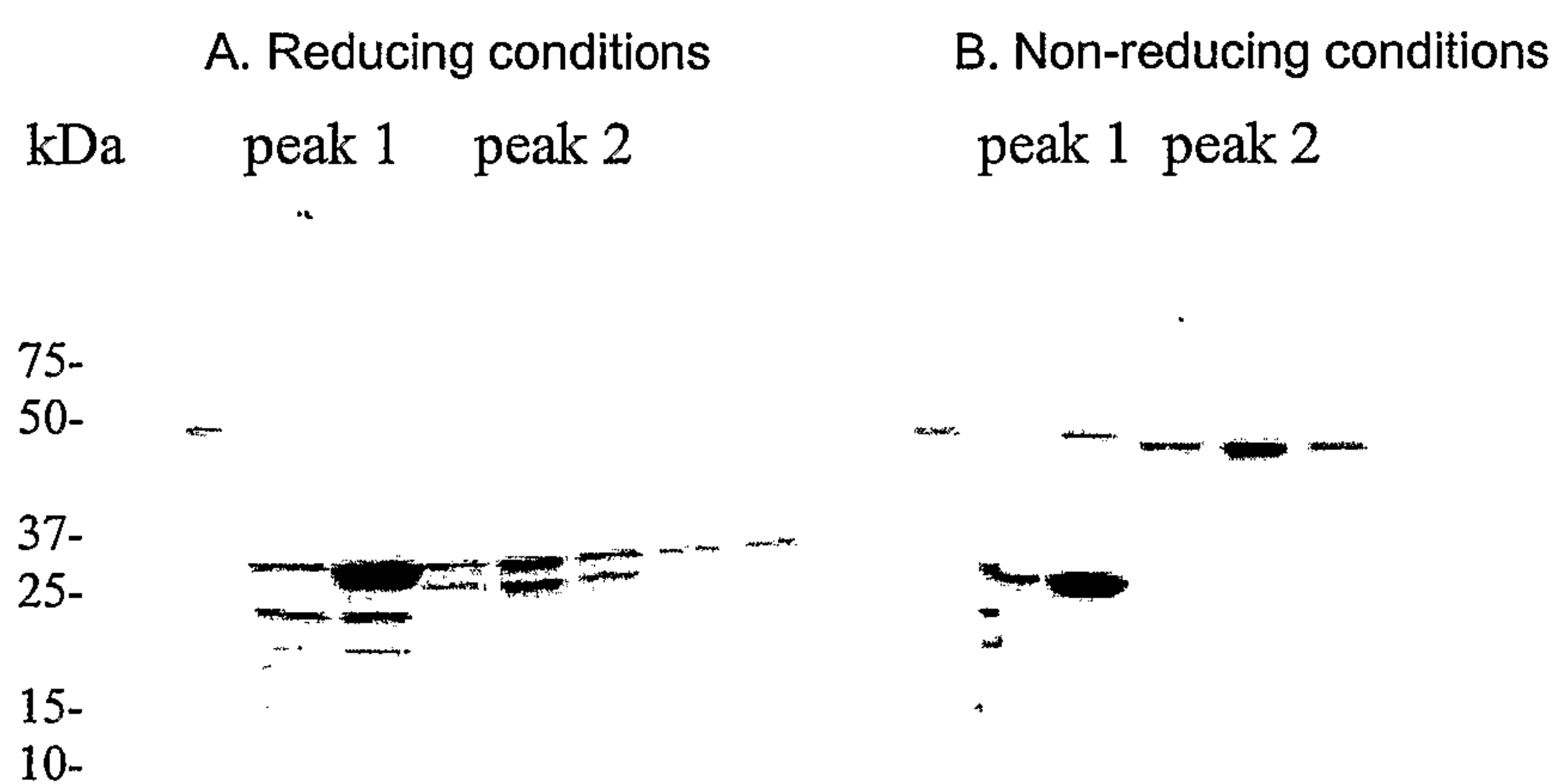
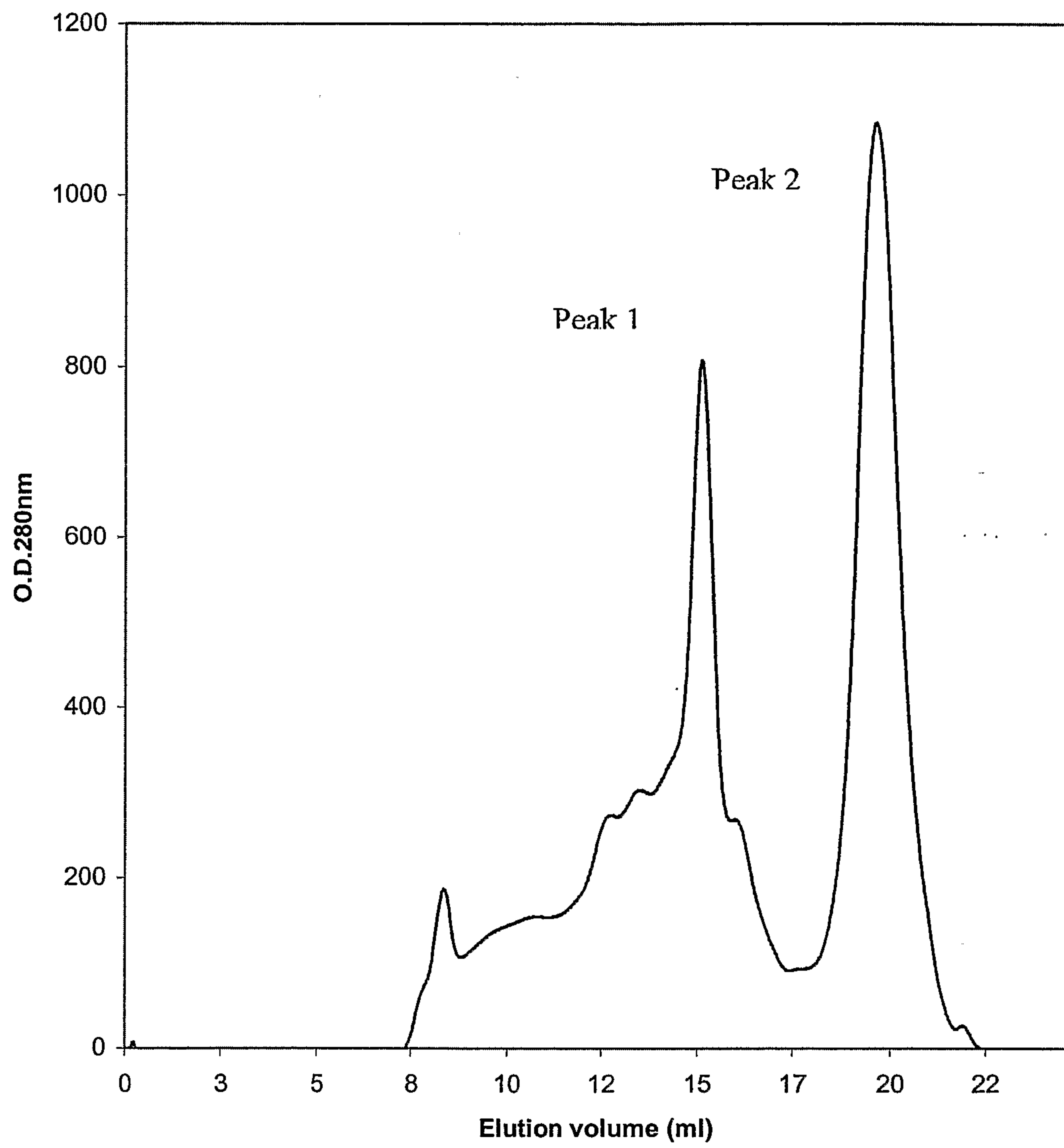


Figure 104



71/81
Figure 105



72/81
Figure 106

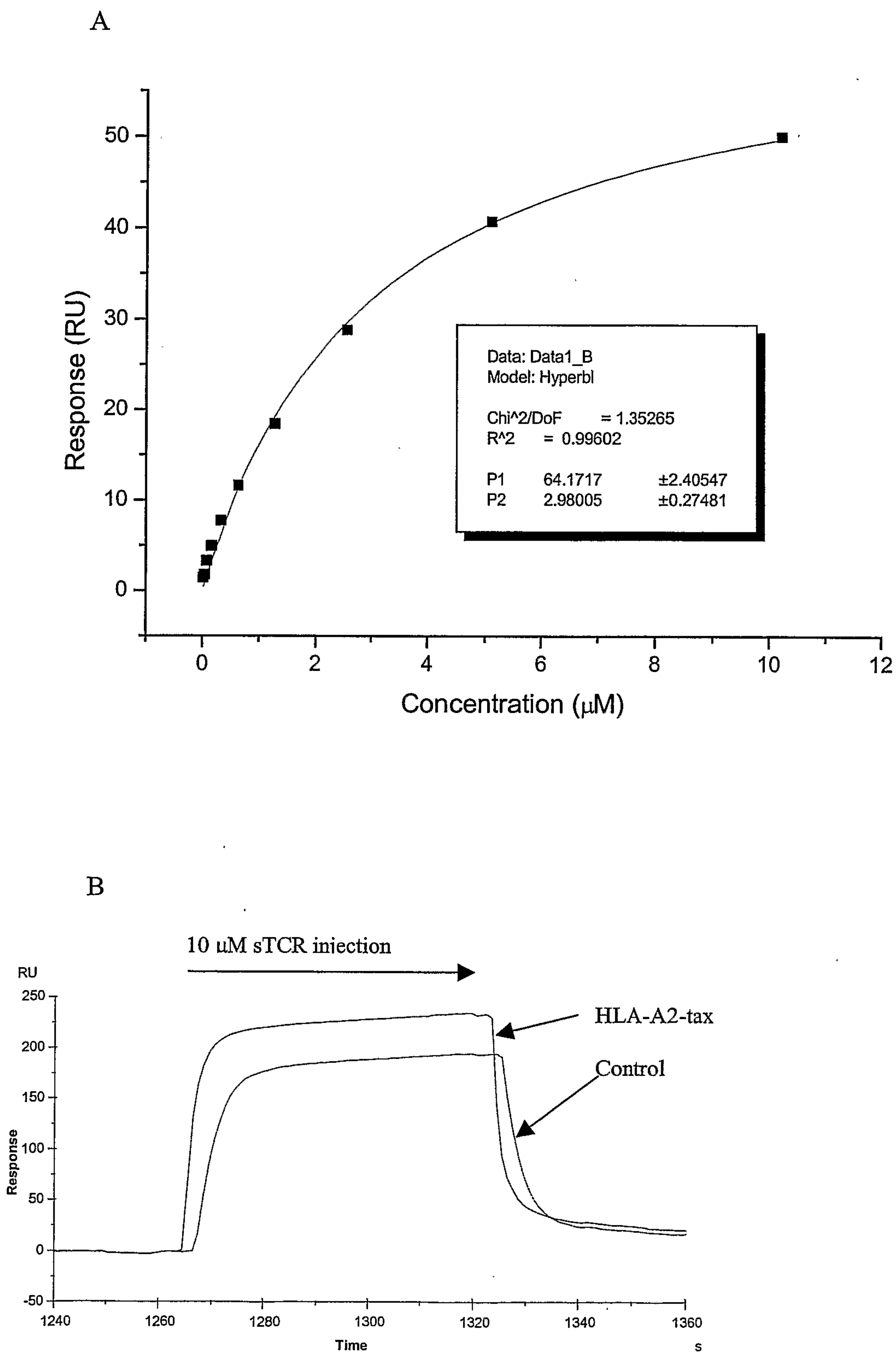


Figure 107

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 201 TTTGTTCCCT TTAGTGAGGG TTAATTCAAT TCACTGGCCG TCGTTTACA ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGAG
 301 CACATCCCCC TTTCCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GCGGAATGGC GCGACGCGCC
 401 CTGTAGCGGC GCATTAGCG CGGCGGGTGT CGGCTTCCC CGTCAAGCTC TAAATCGGG GCTCCCTTTA GGTTCGGAT TTAGTGGTTT ACGGCACCTC GACCCCAAAA
 501 TCCCTTCTCG CCACGTTGCG GGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTTCTTAATA GTGGACTCTT
 601 AACTTGATTA GGTGATGGT GGAACAACAC TCAACCCTAT CTGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCGGATT TCGGCTATT GGTAAAAAA TGAGCTGATT
 701 GTTCCAAACT GGAACAACAC TCAACCCTAT CTGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCGGATT TCGGCTATT TCGGCTATT TCGGCTATT CACACCGCAT
 801 TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATFAACGT TTACAATTTC CTGATGCGGT ATTTCTCTCT TACGCATCTG TCGGCTATT TCGGCTATT CACACCGCAT
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 2801 GAAATGTGCG CGGAACCCCT ATTTGTTTAT TTTTCTAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAAACCGTGA TAAATGCTTC AATAATATTG
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Figure 107 continued

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Figure 108

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Figure 108 continued

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 6601 TTAGGAGTTT AGTGAACCTG CAACATTTAC TATTTTCCCT TCTTACGTAA ATATTTTCT ATATTTTCT TTTTAAATCT AAAATCAATCT TTTTCAATTT TTTGTTTGTG
 6701 TTCCTTTTCTT GCTTAAATCT ATAACTACAA AAAACACATA CAG

Figure 109

MetArg PheProSer IlePheThr AlaValLeuPhe AlaAlaSer SerAlaLeu AlaAlaProVal AsnThrThr ThrGluAsp GluThrAlaGln.
1 GAATTCATGA GATTTCCTTC AATTCTTACG GCGATTCTTA TCGGAGGATG CTGGCCATTA GCTGCTCCAG TCAACACTAG ATCAGAAAGAT GAAAGCGCAG
101 ..IleProAla GluAlaVal IleGlyTyrLeu AspLeuGlu GlyAspPhe AspValAlaVal LeuProphe SerAsnSer ThrAsnAsnGly LeuLeuPhe.
AAATTCGGGG TCAAGCTGTC ATGCGTTACT TACATTACG ACGGATTCG GAGCTTGGC TTCTGGCATT TCCCAACAGC AGAATAAGC GCTTAATTCTT
101 ..IleAsnThr ThrIleAlaSer IleAlaAla LysGluGlu GlyValSerLeu AspLysArg GluAlaGlu AlaGlnGluVal ThrGlnIle ProAlaAla
TATAAATACT ACTATTGGCA GCAATTCGGC TAAAGAACAA GCGCTATCTT TCCATTAATG AGAGGCTGAA GCGCAGGAGG TGACACAGAT TCCTGCAGCT
201 LeuSerValPro GluGlyGlu AsnLeuVal LeuAsnCysSer PheThrAsp SerAlaIle TyrAsnLeuGln TrpPheArg GlnAspPro GlyLysGlyLeu.
CTGAGTGTCC CAGAAGGAGA AAACCTTGTT CTCAACTGCA GTTTCACCTGA TAGCGCTATT TACAACCTCC AGTGGTTTAG GCAGGACCCCT GGGAAAGGTC
301 ..ThrSerLeu LeuLeuIle GlnSerSerGln ArgGluGln ThrSerGly ArgLeuAsnAla SerLeuAsp LysSerSer GlyArgSerThr LeuTyrIle.
TCACATCTCT GTTGCTTATT CAGTCAAGTC AGAGAGAGCA AACAAAGTGA AGACTTAATG CCTCGCTGA TAAATCATCA GGACGTAGTA CTTTATACAT
401 .AlaAlaSer GlnProGlyAsp SerAlaThr TyrLeuCys AlaValArgPro ThrSerGly GlySerTyr IleProThrPhe GlyArgGly ThrSerLeu
TGCAGCTTCT CAGCCTGGTG ACTCAGCCAC CTACCTCTGT GCTGTGAGGC CCACATCAGG AGGAAGCTAC ATACCTACAT TTGGAAGAGG AACCCAGCCTT
501 IleValHisPro TyrIleGln AsnProAsp ProAlaValTyr GlnLeuArg AspSerLys SerSerAspLys SerValCys LeuPheThr AspPheAspSer.
ATTGTTTCATC CGTATATCCA GAACCCGGAT CCTGCCGTGT ACCAGCTGAG AGACTTAAA TCCAGTGACA AGTCTGTCTG CCTATTTCACC GATTTTGATT
601 ..GlnThrAsn ValSerGln SerLysAspSer AspValTyr IleThrAsp LysCysValLeu AspMetArg SerMetAsp PheLysSerAsn SerAlaVal.
CTCAAAACAAA TGTGTACAAA AGTAAGGATT CTGATGTGTA TATCACAGAC AAAATGTGTG TAGACATGAG GTCTATGGAC TTCAAGAGCA ACAGTGCTGT
701 .AlaTrpSer AsnLysSerAsp PheAlaCys AlaAsnAla PheAsnAsnSer IleIlePro GluAspThr PhePheProSer ProGluSer Ser***
801 GGCCTGGAGC AACAAATCTG ACTTTGCATG TGCAAACGCC TTCAACAACA GCATTATTCC AGAAGACACC TTCTTCCCCA GCCCAGAAAG TTCCTAACTC
901 GAG

The pre-pro mating factor alpha sequence is highlighted. BamHI site is underlined.

Figure 110

MetArg PheProSer IlePheThr AlaValLeuPhe AlaAlaSer SerAlaLeu AlaAlaProVal AsnThrThr ThrGluAsp GluThrAlaGln.
1 GAATTCATGA GATTTCTTTC AATTTTACT GCAGTTTAT TGCAGCATC CTCGGCATTA GTGCTCCAG TCAACACTAC AACAGACAT GAAACGGCAG
101 ..IleProAla GluAlaVal IleGlyTyrLeu AspLeuGlu GlyAspPhe AspValAlaVal LeuProphe SerAsnSer ThrAsnAsnGly LeuLeuPhe.
AAATTCCGGC TGAAGCTGTC ATCGGTTACT TACATTAGA AGCGAATTC GATGTTCGTG TTTTGCCATT TTCCAACAGC AGAAATAACG GGTATTGTT
201 ..IleAsnThr ThrIleAlaSer IleAlaAla LysGluGlu GlyValSerLeu AspLysArg GluAlaGlu AlaGlyValThr GlnThrPro LysPheGln
PATAAATACT ACTATTGCCA GCATTGCTGC TAAAGAAGAA GGGCTATCTT TGCATTAAG ACAGGCTGAA GCGGGCTCA CTCAGACCCC AAAATTCCAG
301 ValLeuLysThr GlyGlnSer MetThrLeu GlnCysAlaGln AspMetAsn HisGluTyr MetSerTrpTyr ArgGlnAsp ProGlyMet GlyLeuArgLeu.
GTCCTGAAGA CAGGACAGAG CATGACACTG CAGTGTGCCC AGGATATGAA CCATGAATAC ATGTCCTGGT ATCGACAAGA CCAGGCATG GGGCTGAGGC
401 ..IleHisTyr SerValGly AlaGlyIleThr AspGlnGly GluValPro AsnGlyTyrAsn ValSerArg SerThrThr GluAspPhePro LeuArgLeu.
TGATTCAATTA CTCAGTTGGT GCTGGTATCA CTGACCAAGG AGAAGTCCC AATGGCTACA ATGCTCCAG ATCAACCACA GAGGATTTC CGCTCAGGCT
501 .LeuSerAla AlaProSerGln ThrSerVal TyrPheCys AlaSerSerTyr ValGlyAsn ThrGlyGlu LeuPhePheGly GluGlySer ArgLeuThr
GCTGTGGGCT GCTCCCTCCC AGACATCTGT GTACTTCTGT GCCAGCAGTT ACGTCGGGAA CACCGGGGAG CTGTTTTTTG GAGAAGGCTC TAGGCTGACC
601 ValLeuGluAsp LeuLysAsn ValPhePro ProGluValAla ValPheGlu ProSerGlu AlaGluIleSer HisThrGln LysAlaThr LeuValCysLeu.
GTAATGGAGG ACCTGAATAA CGTGTTCCTCA CCCGAGGTCG CTGTGTTTGA GCCATCAGAA GCAGAGATCT CCCACACCCA AAAGGCCACA CTGGTGTGCC
701 ..AlaThrGly PheTyrPro AspHisValGlu LeuSerTrp TrpValasn GlyLysGluVal HiserGly ValCysThr AspProGlnPro LeuLysGlu.
TGGCCACAGG CTTCTACCCC GACCACGTGG AGCTGAGCTG GTGGGTGAAT GGGAAGGAGG TGCACAGTGG GGTCTGCACA GACCCGCAGC CCTCAAGGA
801 .GlnProAla LeuAsnAspSer ArgTyrAla LeuSerSer ArgLeuArgVal SerAlaThr PheTrpGln AspProArgAsn HisPheArg CysGlnVal
GCAGCCCGCC CTCAATGACT CCAGATACGC TCTGAGCAGC CGCCTGAGGG TCTCGGCCAC CTTCTGGCAG GACCCCGCA ACCACTTCCG CTGTCAAGTC
GlnPheTyrGly LeuSerGlu AsnAspGlu TrpThrGlnAsp ArgAlaLys ProValThr GlnIleValser AlaGluAla TrpGlyArg Alaasp**
901 CAGTTCTACG GGCTCTCGGA GAATGACGAG TGGACCCAGG ATAGGGCCAA ACCCGTCACC CAGATCGTCA GCGCCGAGGC CTGGGGTAGA GCAGACTAAC
1001 TCGAG

The pre-pro mating factor alpha sequence is highlighted.

Figure 111

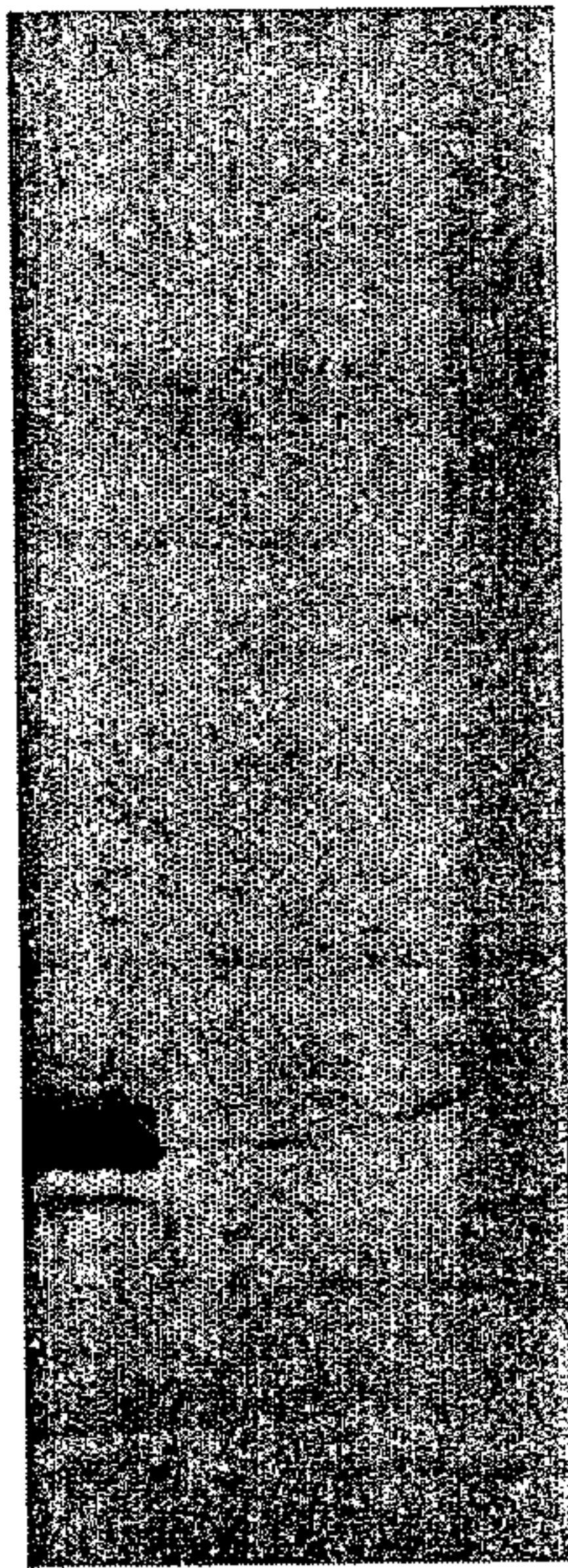


Figure 112

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Figure 113

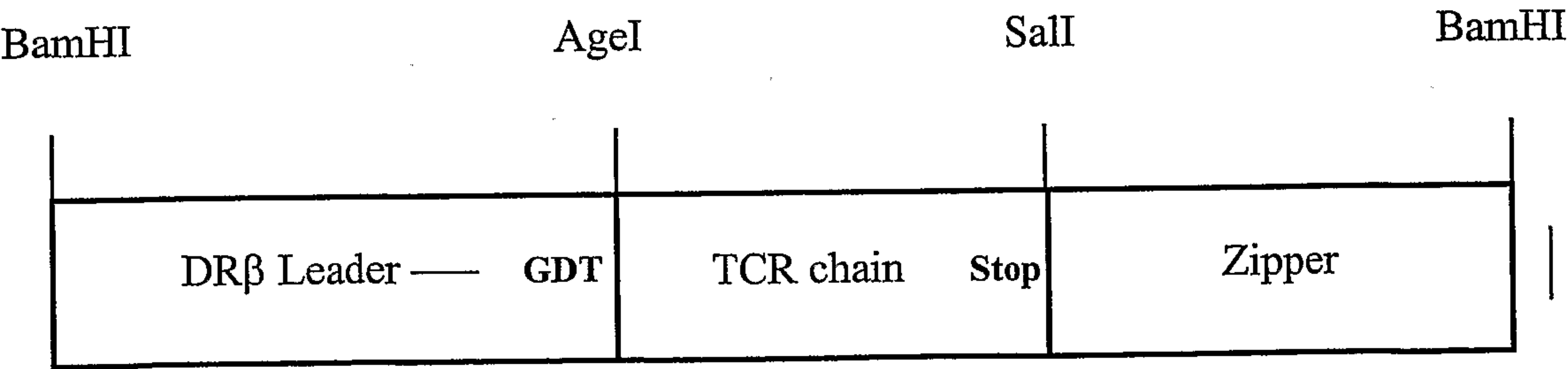


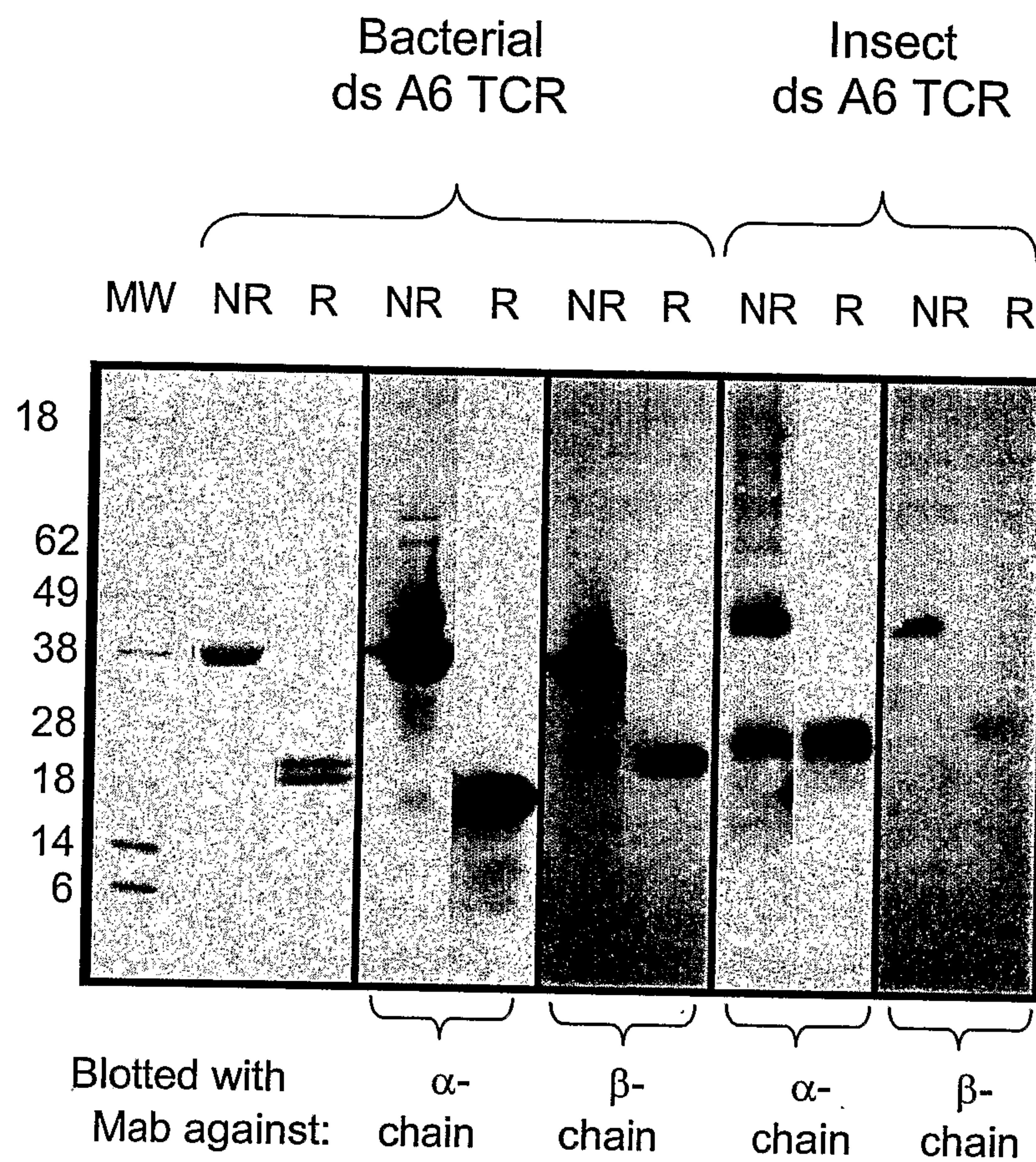
Figure 114

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Figure 115

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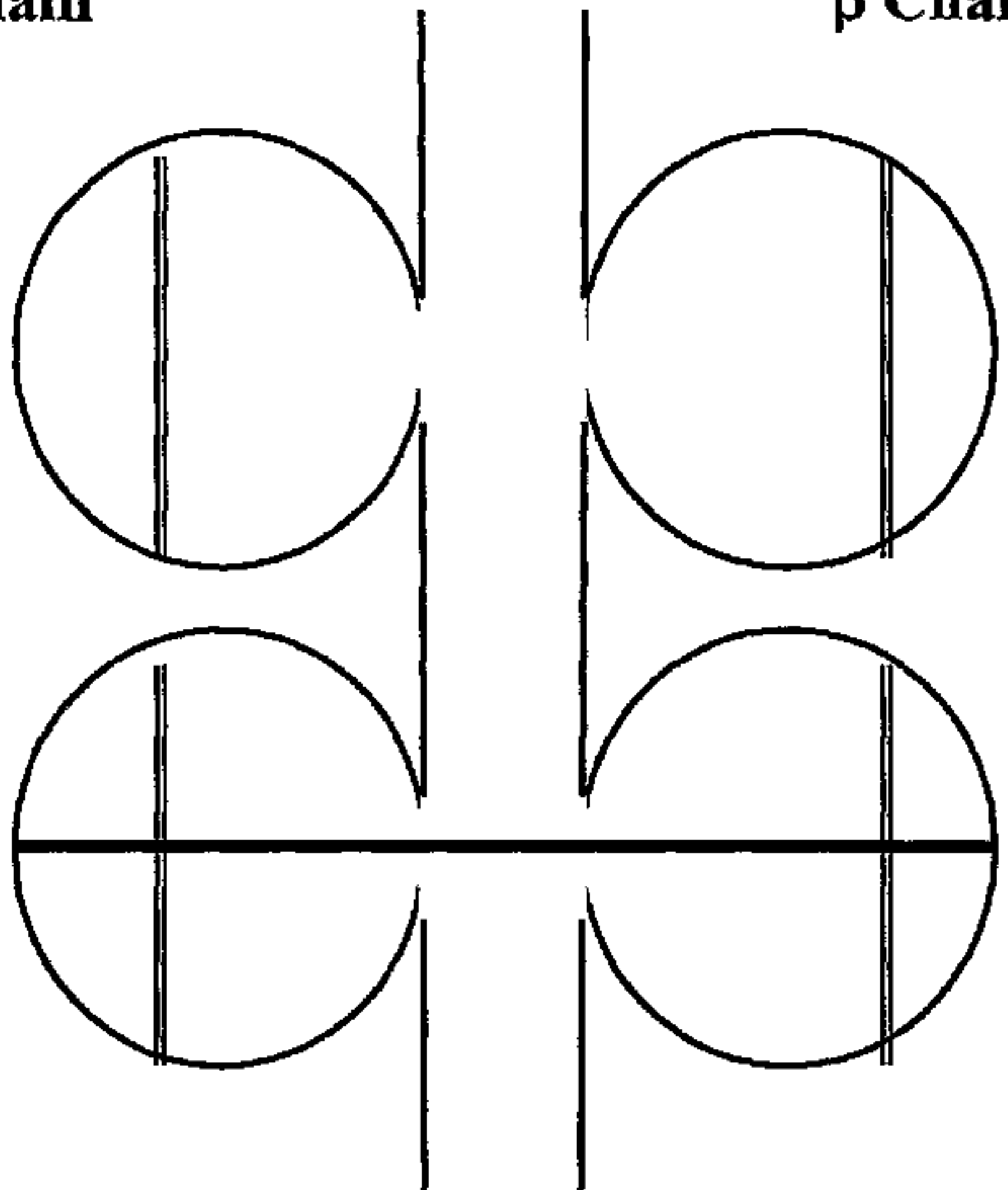
Figure 116



Key:
R: Reduced
NR: Non-reduced

α Chain

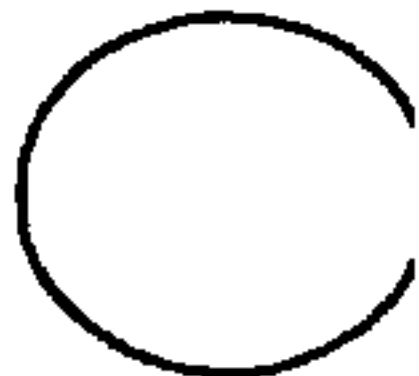
β Chain



Native intra-chain disulphide bond



Non-native interchain disulphide bond



TCR domain