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(54) Titre : ELEMENT DE LA CLASSE II DU COMPLEXE MAJEUR D'HISTOCOMPATIBILITE PORTANT UN
EPITOPE/MOLECULES CHIMERES D'IMMUNOGLOBULINE
(54) Title: EPITOPE-BEARING MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II ELEMENT/IMMUNOGLOBULIN
CHIMERIC MOLECULES

(57) **Abrégé/Abstract:**

The present invention relates to immunologically active molecules comprising an epitope of interest, more than one major histocompatibility complex class II element, and an immunoglobulin constant region element, wherein the epitope is comprised in a fusion protein which comprises a major histocompatibility class II element, wherein each major histocompatibility class II element comprises two non-covalently associated chains comprising extracellular domains of a major histocompatibility complex class II protein, and wherein the major histocompatibility class II elements are covalently joined by one or more disulfide linkages present in the immunoglobulin constant region element. The molecules of the invention may be used to selectively eliminate T cells bearing TCRs which react with the epitope of interest in the context of the major histocompatibility complex class II element, and therefore may be used to eliminate or reduce specific T cell populations, for example, but not by way of limitation, in the treatment of an autoimmune disease and/or a graft-versus host disease.

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(54) Title: EPITOPE-BEARING MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II ELEMENT/IMMUNOGLOBULIN CHIMERIC MOLECULES (57) Abstract The present invention relates to immunologically active molecules comprising an epitope of interest, more than one major histocompatibility complex class II element, and an immunoglobulin constant region element, wherein the epitope is comprised in a fusion protein which comprises a major histocompatibility class II element, wherein each major histocompatibility class II element comprises two non-covalently associated chains comprising extracellular domains of a major histocompatibility complex class II protein, and wherein the major histocompatibility class II elements are covalently joined by one or more disulfide linkages present in the immunoglobulin constant region element. The molecules of the invention may be used to selectively eliminate T cells bearing TCRs which react with the epitope of interest in the context of the major histocompatibility complex class II element, and therefore may be used to eliminate or reduce specific T cell populations, for example, but not by way of limitation, in the treatment of an autoimmune disease and/or a graft-versus host disease.		

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DescriptionEpitope-Bearing Major Histocompatibility Complex
Class II Element/Immunoglobulin Chimeric Molecules

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1. INTRODUCTION

The present invention relates to immunologically active molecules comprising an epitope of interest, more than one major histocompatibility complex class II element, and an immunoglobulin constant region element.

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2. BACKGROUND OF THE INVENTION

The function of the immune system is to remove pathogens and eliminate diseased cells; this process is only effective if antigens associated with offending cells and microbes are distinguishable from antigens normally present in the body. The distinction between self and non-self is effected, at least in part, by the interaction of classes of molecules present on cellular elements of the immune system, including B lymphocytes, T lymphocytes, and professional antigen presenting cells ("APCs") such as macrophages and dendritic cells. One such interaction is between the T cell receptor ("TCR") present on the surface of T lymphocytes and elements of the major histocompatibility complex ("MHC"). In order to respond to a non-self antigen, a T cell must encounter the antigen in the context of a self MHC molecule.

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There are two classes of MHC molecules, referred to as "class I" and "class II" antigens. Cytotoxic T lymphocytes (which bear CD8 surface antigen, and are referred to as "CD8⁺ T lymphocytes") will only kill cells bearing a foreign antigen in the context of a self MHC class I molecule. Analogously, helper T lymphocytes (which bear CD4 surface antigen, and are referred to as "CD4⁺ T lymphocytes") will only proliferate in response to a foreign antigen in the context of a self MHC class II molecule on the surface of an APC (for review, see Davies, H., 1997, *Introductory Immunobiology*, Chapman & Hall, New York, pp. 177-223). While class I molecules are comprised of a

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heavy α chain and a β_2 -microglobulin light chain, class II molecules are heterodimers comprised of α and β chains, each having two domains and being of approximately the same length. The DNA regions containing MHC genes have been well characterized for mouse and man, the mouse MHC being referred to as the "H-2 complex" and the human MHC being referred to as the "HLA complex" (for Human Leukocyte Antigen). Class I molecules are encoded at the A, B and C loci in man and the K, D, and L loci in mouse. Class II molecules are encoded at the DP, DQ and DR regions in man and the I-A and I-E regions in mouse. At each region, a multitude of alleles have been identified.

While the combined interaction between T cells, MHC-peptide complexes and costimulatory molecules on the surface of APCs induces T cell activation, the absence of costimulatory molecules induces T cell unresponsiveness. The latter seems to account for the peripheral self-tolerance of tissue-specific antigens (Guerder et al., 1995, Int. Rev. Immunol. 13:135-146). Based on this knowledge, soluble antigen presenting molecules such as (i) MHC molecules extracted from cell membranes and subjected to peptide elution followed by exchange for specific peptides *in vitro* (Nag et al., 1996, Cell. Immunol. 170:25-33; Sharma et al., 1991, Proc. Natl. Acad. Sci. U.S.A. 88:11465-11469; Spack et al., 1995, J. Autoimmunity 8:787-807); (ii) recombinant MHC molecules loaded with peptides *in vitro* (Abastado et al., 1995, J. Exp. Med. 182:439-447; Altman et al., 1996, Science 274: 94-96; Godeau et al., 1992, J. Biol. Chem. 267:24223-24229; Scheirle et al., 1992, J. Immunol. 149:1994-1999; Scott et al., 1996, J. Exp. Med. 183:2087-2095; Stern and Wiley, 1992, Cell 68:465-477), or (iii) genetically engineered, covalently-linked peptide /MHC chimeras (International Application No. PCT/US95/02689 (WO 95/23814) by Kappler and Marrack; Kozono et al., 1994, Nature 369:151-154; Mottez et al., 1995, J. Exp. Med. 181:493-502; Rhode et al., 1996, J. Immunol. 15:4885-4891), became a platform for designing prodrugs with potential immunomodulatory effects. However, because self peptides bound to the MHC groove exhibit very low off rates (Buus et al., 1987, Immunol. Rev. 98:115-141, Tampe et al., 1991, Proc. Natl. Acad. Sci. U.S.A. 88:4661-4665), the generation of newly homogeneous populations of MHC-peptide complexes *in vitro* for therapeutic use in humans has proved difficult.

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Another drawback of recombinant MHC/peptide complexes which lack any membrane anchoring motif resides in their intrinsic low affinity interaction with TCRs (Corr et al., 1994, Science 265:946-949; Matsui et al., 1994, Proc. Natl. Acad. Sci. U.S.A. 91:12862-12866; Sykulev et al., 1994, Immunity 1:15-22; Weber et al., 1992, Nature 356:793-796). Several investigators have produced non-covalently multimerized MHC class I /peptide complexes in order to improve binding to the TCR. A human HLA-A2 heavy chain MHC molecule expressing the BirA-dependent biotinylation site at its carboxy terminus was tetramerized by streptavidin and used to phenotype low frequency HIV-specific T cells in the blood of HIV-infected individuals (Altman et al., 1996, Science 274:94-96). A similar approach showed that soluble MHC class I/peptide complexes dimerized by a polyclonal anti-class I MHC antibody (but not the corresponding monomers) were able to bind with high affinity to antigen-specific T cells (Abastado et al., 1995, J. Exp. Med. 182:439-447). The stability of these non-covalently associated monomers remains, however, problematic. In International Application No. PCT/US92/10030 (WO 93/10220) by Selick and Armstrong, chimeric molecules comprising class I or class II MHC molecules were constructed and subsequently loaded with antigenic material, which could be non-covalently bound or crosslinked to the chimera. Here, the formation of appropriate interactions between MHC and antigenic elements may be problematic.

3. SUMMARY OF THE INVENTION

The present invention relates to immunologically active molecules comprising an epitope of interest, more than one MHC class II element, and an immunoglobulin constant region element, wherein the epitope is comprised in a fusion protein which comprises a MHC element, wherein each MHC class II element comprises two non-covalently associated chains comprising extracellular domains of a MHC class II protein, and wherein the MHC class II elements are covalently joined by one or more disulfide linkages present in the immunoglobulin constant region element. The molecules of the invention may be used to selectively eliminate T cells bearing TCRs which react

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with the epitope of interest in the context of the major histocompatibility complex class II element, and therefore may be used to eliminate or reduce specific T cell populations, for example, but not by way of limitation, in the treatment of an autoimmune disease and/or a graft-versus host disease.

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4. DESCRIPTION OF THE FIGURES

FIGURE 1. Gene fragments of the HA110-120/IE^dβ/Fcγ2a chimeric gene after cloning in the p2Bac vector. Gene fragments are shown by digestion with the restriction enzymes used for cloning and analyzed in a 1% agarose gel. Lanes 1 and 2 show low and high molecular weight markers (ϕX174/HaeIII and λ/HindIII, respectively); lane 3 shows a band of 194 bp which corresponds to the cloned fragment containing sequences of the I-E^dβ1-F leader/HA110-120/linker, obtained by amplification with I-E^dβ1-F and I-E^dβ1-R primers and digested with EcoRI/BamHI enzymes; lane 4 shows a band of 609 bp corresponding to the cloned fragment containing sequences of linker/I-E^dβ1/I-E^dβ domains obtained by amplification with I-E^dβ2-F and I-E^dβ2-R primers and digested with BamHI/ApaI enzymes. The lower band corresponds to the fragment present in lane 1 due to the existence of a BamHI site in the multiple cloning site of p2Bac upstream to the cloned sequences; lane 5 shows a band of 699 bp corresponding to a cloned fragment containing sequences of the hinge-CH1-CH2 domains of the Ig-Fc region obtained by amplification with Fc-F and Fc-R primers and digested with ApaI/HindIII enzymes; and lane 6 shows a band of 1502 bp corresponding to the entire HA110-120/I-E^dβ/Fcγa chimeric gene, cloned in the p2Bac vector and digested with EcoRI/HindIII enzymes,

FIGURE 2A-B. Scheme for the genetic construction of a DEF chimeric molecule. (A) sets forth the nucleotide sequence (with gaps, SEQ ID NO:1-6) of the HA110-120/I-E^dβ/FCγ2a gene. Restriction sites are underlined; amino acids (SEQ ID NO:7-10) are indicated below codons. (B) diagrams the cloning of I-E^dα and HA110-120/I-E^dβ/FCγ2a genes under the p10 and pPolH promoters, respectively, of a p2Bac baculovirus vector.

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FIGURE 3A-B. **Chromatographic analyses of the DEF molecule.** (A) depicts the results of size exclusion chromatography on a SuperoseTM 6 column of affinity purified DEF molecules. Dotted peaks indicate the elution time of the molecular size standard proteins; bovine IgG, chicken ovalbumin and cytochrome c. The peak eluting at 170 kDa represents the DEF molecule. (B) depicts the results of anion-exchange chromatography on a MONO Q column of the affinity purified DEF molecules. Both minor peak eluted in 40 mM NaCl and the major peak eluted between 200 and 300 mM gradient of NaCl consisted in a 170 kDa dimer of the HA110-120/I-E^dαβ/Fcγ2a molecule, according to SDS-PAGE and Western blot analyses.

FIGURE 4A-B. **SDS-PAGE and Western blot analyses of the DEF molecule.** SDS-PAGE was performed on 4-12 percent polyacrylamide gradient gels, according to the standard procedure of Laemmli. (A) represents the DEF molecule, as revealed by silver stain; lane 1, no reduction and no boiling; lane 2, no reduction with boiling; lane 3, reduction without boiling; and lane 4, reduction with boiling. (B) represents the Western blot analysis after transferring the gels onto PVDF membranes: lane 1 and 2, DEF molecule under no reducing/no boiling conditions and under reducing and boiling conditions, respectively, as revealed with ¹²⁵I-goat anti-mouse γ2a Abs; lanes 3 and 4, DEF molecule under no reducing/no boiling conditions and under reducing/boiling conditions, respectively, as revealed with ¹²⁵I-14-4-4 mAb.

FIGURE 5A-I. **Cytofluorometric analyses of DEF molecule.** Panels A, D and G indicate the fluorescence background of the developing antibody (goat anti-γ2a-FITC conjugate); panel B indicates binding of DEF molecule (10 μg/ml) to 14.3d TCR of 14-3-1 TcH as revealed by the goat anti-γ2a-FITC conjugate; panel C depicts inhibition of binding of DEF molecule (10 μg/ml) to B_{long} melanoma cells as revealed by goat anti-γ2a-FITC conjugate; panel F shows inhibition of binding of DEF molecule (10 μg/ml) by 2.4G-2 mAb (100 μg/ml); panel H depicts binding of 14-4-4 mAb (10 μg/ml) to B_{long} cells as revealed by goat anti-γ2a-FITC conjugate; and panel I depicts inhibition of binding of 14-4-4 mAb (10 μg/ml) by 2.4G-2 mAb (100 μg/ml).

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FIGURE 6. The genes in the major histocompatibility complex in the mouse.

FIGURE 7. The genes in the major histocompatibility complex of the human on chromosome 6.

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5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to immunologically active molecules comprising an epitope of interest, more than one major histocompatibility complex class II element, and an immunoglobulin constant region element. The molecules of the invention are referred to herein as "DEF" molecules, as they are Disulfide-linked and comprise an Epitope as well as an immunoglobulin constant region (Ec) element.

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The DEF molecules of the invention comprise at least one subunit wherein the epitope of interest is comprised in a fusion protein which also comprises a MHC element (*i.e.*, the epitope of interest and the MHC element are expressed as part of the same protein, and are translated from the same RNA molecule).

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The term "epitope of interest", as used herein, refers to a molecular structure which serves as the part of an antigen which is recognized by an antigen receptor, such as a TCR. The epitope is "of interest" if it is part of an antigen associated with an infectious agent, a self antigen relevant to an autoimmune disease or alloreactive epitopes associated with graft rejection or graft versus host (GVH) disease. There is no limitation as to the epitopes which may be used according to the invention, provided that the epitope of interest is able to associate with the MHC elements of the construct so as to be able to bind to a T cell receptor (such binding may be evaluated, for example, by fluorescence activated cell sorting analysis ("FACS"; see Section 6.1, below).

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Accordingly, issues such as electrostatic charge, hydrophobicity, hydrophilicity, and steric hindrance should be considered when designing the molecule. In certain cases, variation of peptide linkers and other regions of the DEF molecule may be used to optimize orientation of the epitope of interest and MHC elements. Examples of epitopes of interest which may be incorporated into DEF molecules according to the invention

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include those derived from glutamic acid decarboxylase 65 (associated with insulin dependent diabetes mellitus); myelin basic protein (associated with multiple sclerosis); human cartilage glucoprotein 39 (associated with rheumatoid arthritis); wheat gliadin (associated with celiac disease); and acetyl choline receptor (associated with myasthenia gravis).

The term "MHC class II element" refers to a MHC class II molecule or portions thereof which is a functional antigen presenting molecule; as such, the MHC class II element preferably comprises an α chain component and a β chain component. The α and β chain components preferably comprise all or part of the extracellular domains of the complete α and β chain proteins. The MHC class II element may be human or non-human, and is selected to be of the same species as the intended recipient of the DEF molecule. Examples of human MHC class II elements include DP, DQ and DR molecules and portions thereof, for which numerous alleles are known. Particularly preferred are MHC class II elements associated with particular autoimmune conditions: for example, DR3, DQw2 and DR4, DQw3 (associated with insulin dependent diabetes mellitus (IDDM)); DR4, DQw3 and DR1, DQw1 (associated with rheumatoid arthritis); DR2, DQw1 (associated with multiple sclerosis); DR3, DQw2 and DR7, DQw2 (associated with celiac disease); DR4, DQw3, and DR6, DQw (associated with pemphigus vulgaris); DR8 and DR5 (associated with pauciarticular juvenile rheumatoid arthritis); DR3, DQw2, and DR2, DQw1 (associated with systemic lupus erythematosus); DR3 (associated with Sjogren's syndrome); DR2, DQw1 (associated with narcolepsy), DR3, DQw2 (associated with Graves' disease); and DR3, DQw2 (associated with dermatitis herpetiformis).

The term "immunoglobulin constant region element" refers to a portion of an immunoglobulin molecule comprising all or part of the C-terminal portion of both heavy chains wherein one heavy chain constant region component is joined to the other by one or more disulfide bond. The immunoglobulin constant region element is alternatively referred to herein as an Fc element, even though the term is not herein restricted to the product resulting from papain digestion of an immunoglobulin molecule.

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Preferably, the immunoglobulin constant region element comprises all or a portion of the hinge region, as the hinge region may comprise the required disulfide bond.

The DEF molecule of the invention comprises four protein chains, wherein two heterodimers are joined together via one or more disulfide bond in the immunoglobulin constant region element. Each heterodimer comprises (i) a first protein chain which itself comprises the immunoglobulin constant region element linked to a MHC class II α or β chain element and (ii) a second protein chain which comprises the complementary (α or β) chain element; the first or second chain or both may comprise the epitope of interest. The DEF molecule of the invention is preferably glycosylated. For example, but not by way of limitation, the DEF molecule of the invention comprises (a) two MHC class II elements; (b) an immunoglobulin constant region element comprising two protein chains covalently joined by a disulfide linkage; and (c) an epitope of interest, wherein each of the protein chains of the immunoglobulin constant region element is covalently joined to one of the two MHC class II elements by a peptide bond.

In specific nonlimiting embodiments of the invention, nucleic acids encoding a DEF molecule may be prepared as follows. Because the final DEF molecule produced according to the invention is a tetramer composed of two pairs of distinct DEF subunits (referred to hereafter as the "A DEF subunit" and the "B DEF subunit", where two noncovalently linked dimers (A-B) are covalently linked via the B DEF subunit to form the tetramer (A-B=B-A)), one or both subunits may comprise the desired epitope. The B DEF subunit is encoded by a nucleic acid sequence comprising regions encoding both MHC subunit and Fc elements; to produce such a nucleic acid construct, nucleic acids encoding (i) one or more external domains of a first subunit of an MHC molecule and optionally an epitope of interest and (ii) a constant (Fc) region of an immunoglobulin molecule may be prepared separately and then ligated together. The other DEF-encoding construct needed to produce the complete DEF molecule (the A DEF subunit) may comprise sequence encoding one or more external domains of a second MHC subunit, and may optionally further comprise sequence encoding an epitope of interest. Constructs encoding the A and B DEF subunits may then be translated into the corresponding

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proteins in an appropriate expression system. To form the complete DEF molecule, a protein dimer between the A and B DEF subunits is formed when the domains of the first and second MHC subunits noncovalently bind (A-B), and the complete tetramer is formed when the Fc portions of the B DEF subunits are covalently joined via disulfide linkages (A-B=B-A).

For example, as a first step, where the sequence of an MHC class II gene is known, oligonucleotide primers may be designed so that nucleic acid sequence encoding one or more external domains of a first MHC subunit may be obtained by reverse transcription - polymerase chain reaction ("RT-PCR") of mRNA obtained from cells expressing the MHC class II gene (for example, a lymphoma cell line). In particular, oligonucleotide primers used in the RT-PCR reaction may be designed to (optionally) incorporate nucleic acid sequence encoding the epitope of interest, nucleic acid sequence encoding other peptide linkers, where appropriate, and/or nucleic acid sequence containing one or more restriction enzyme cleavage site(s) that facilitate the splicing together of MHC and Fc regions. Nucleic acid encoding at least a portion of the Fc hinge region may also be included in a primer, particularly where such nucleic acid encodes a restriction enzyme cleavage site. The primers should also be designed such that nucleic acid and/or encoded peptide sequences necessary or desirable for the proper processing of the final DEF product are included, such as, but not limited to, a leader sequence and polyadenylation sequences.

Of note, nucleic acid encoding the epitope of interest may be introduced into constructs encoding the A and/or B DEF subunit using genetic engineering techniques, at any point within the MHC gene according to the invention; incorporation into an oligonucleotide primer used in RT-PCR is suggested above because first, it is most convenient, and second, because it results in a DEF molecule having the epitope in a position where it has greater flexibility to associate with the MHC domains. Such flexibility may be further improved by providing for a peptide linker between the epitope and the MHC region; adjusting the length of the peptide linker may optimize epitope/MHC interactions.

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References disclosing the nucleic acid sequences of various MHC class II genes and cell lines expressing the corresponding MHC class II molecules are listed in GENE BANK and Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. Sequences of proteins of immunological interest. 4th Ed. 1987, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health.

See also FIGURES 6 and 7.

Further, as a second step, nucleic acid encoding all or a portion of an Fc region of an immunoglobulin molecule may be prepared. Such nucleic acid preferably comprises a region encoding the hinge portion of an immunoglobulin molecule. As set forth above, nucleic acid encoding such Fc regions may be obtained by RT-PCR of mRNA obtained from cell lines expressing an immunoglobulin comprising the desired Fc region. Primers for performing such a RT-PCR reaction may be designed using known Fc sequences. The foregoing GENBANK and Kabat references include a series of references providing the sequences of various Fc regions, as well as cell lines expressing said Fc regions. The primers may preferably be designed to comprise one or more restriction enzyme cleavage site to facilitate ligation to nucleic acid encoding the MHC portion of the molecule and incorporation into a suitable vector.

As a third step, nucleic acid sequences encoding the MHC and Fc portions of the desired DEF molecule may be joined (for example, by cleavage at specific sites using one or more restriction enzymes followed by ligation) and introduced into an appropriate expression vector. Suitable expression vectors include prokaryotic expression vectors such as pRSET, pTrcHis, pSE420, pSE380, and PSE280; baculovirus expression vectors such as pVL1392/3, pBlueBacHis, p2Bac, pAC360, and pBluBacIII; eucaryotic expression vectors such as pCMV/EBNA, λ PopTM6, pREP4, pCEP4, pREP7, pREP8, pREP9, pREP10, pEBVHis, PRC/CMV, pRC/RSV, pcDNA3, pcDNAI/Amp, pcDNAI, pCDM8; and yeast expression vectors such as pYES2; suitable expression systems include mammalian cells such as COS cells, insect cells (e.g. the baculovirus system), yeast cells (e.g., Pichia, Hanensula), and bacterial cells, but bacterial cells are least preferred as they are less likely to produce properly processed DEF molecules.

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Mammalian cells, while most likely to produce a properly processed molecule, may give low yields of recombinant protein. A particularly preferred expression vector is p2Bac (Invitrogen) for use in the baculovirus infection of SF9 insect cells in culture. The expression vector preferably comprises elements necessary and/or desirable for the expression of the DEF subunit, including promoter/enhancer sequences, appropriate transcription and translation signals, polyadenylation sites, Shine Delgarno sequences, etc..

Fourth, a DEF construct encoding the A DEF subunit, which comprises one or more external domains of a second (complementary) MHC subunit may be prepared using similar techniques, which may or may not comprise an epitope of interest. In alternative embodiments of the invention, the two DEF constructs used to produce the complete DEF molecule may either be contained in the same or in separate expression vectors. In a preferred, nonlimiting embodiment of the invention, both A and B DEF subunit encoding nucleic acid constructs are incorporated into the same expression vector, so that they are produced simultaneously, thereby allowing the DEF molecule to assemble. Alternatively, separate expression vectors may either be cointroduced into a cell for protein synthesis (for example, by cotransfection where markers allow for the selection of cells containing both constructs), or the A and B DEF subunits may be produced separately and then allowed to associate into the desired tetramers.

Molecular weight, degree of glycosylation, and disulfide linkage of the resulting DEF molecule may be evaluated and confirmed by standard techniques (see, for example, Example Section 6, below).

In alternative embodiments of the invention, a DEF molecule may be produced using methods similar to those set forth above except that the epitope of interest may not be produced as part of one or both A and B DEF subunits during transcription and translation of the molecule. Therefore, the nucleic acids encoding the A and B DEF subunits do not encode the epitope of interest; instead, the epitope of interest may be added to the molecule later, for example, by chemical conjugation or by association *in vitro* or *in vivo*. In specific, nonlimiting embodiments of the invention, the epitope of

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interest may be conjugated to a parent DEF molecule (which lacks the epitope of interest (the "E" component) but is nonetheless referred to herein as a DEF molecule to avoid overly burdensome nomenclature) by linkage to amino acid or carbohydrate components (see, for example, Brumeanu et al., 1995, J. Immunol. Methods 183:185-197; Brumeanu et al., 1996, Nature Biotech. 14:722-725; Brumeanu et al., 1997, Eur. J. Immunol. 27:2408-2416). Such conjugation may be performed on a tetrameric DEF molecule or on any monomeric or dimeric component thereof. Where broad subsets of T cells are desired to be eliminated, DEF molecules lacking any discrete epitope of interest may also be used according to the invention.

10 The DEF molecules of the invention may be further modified to adjust their biological properties according to their intended purpose. For example, but not by way of limitation, half-life may be increased by pegylation of either amino acid or carbohydrate residues (see, for example, Brumeanu et al., 1995, J. Immunol. Methods 183:185-197; Brumeanu et al., 1996, Nature Biotech. 14:722-725; Brumeanu et al., 1997, Eur. J. Immunol. 27:2408-2416). As another example, the DEF molecule may be covalently or noncovalently linked to another molecule having biological activity which may act as a cytokine or as a toxin; exemplary cytokines include TGF β , IL-4, IL-5, IL-10 and exemplary toxins include cyclosporin, steroids or activators of apoptosis such as ICE or FASL.

20 The present invention further provides for a method of treating an autoimmune disease or of treating or preventing a graft versus host disease comprising administering, to a subject in need of such treatment, an effective amount of a DEF molecule according to the invention. Examples of such autoimmune diseases include, but are not limited to, rheumatoid arthritis including juvenile and adult forms, diabetes, multiple sclerosis, systemic lupus erythematosus, scleroderma, sjogren's syndrome, celiac Disease, pemphigus vulgaris, narcoelpsy, Graves' Disease and Dermatitis Herpetiformis. The term "treating", as used herein, does not necessarily imply that the method achieves a complete cure, but rather that the subject exhibits a significant degree of clinical improvement as judged by symptoms and/or clinical signs. Where a subject is to be

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5 treated for an autoimmune disease, the choice of MHC class II element comprised in the DEF molecule may be such that the MHC class II element is syngeneic with an MHC class II protein expressed on the APCs of the subject to which it will be administered; for example, DEF comprising an MHC class II element corresponding to the HLA-DR2 allele may be administered to a subject whose APCs express HLA-DR2, etc.. Where a subject is to be treated to ameliorate or prevent graft versus host disease, the MHC class II element comprised in the DEF molecule may be such that the MHC class II element is syngeneic with an MHC class II protein expressed by APCs of the graft donor. The invention provides for the manufacturing of pharmaceutical compositions for use in the treatment of the foregoing conditions.

6. **EXAMPLE: ENGINEERING AND CHARACTERIZATION OF A MURINE MHC CLASS II-IMMUNOGLOBULIN CHIMERA EXPRESSING AN IMMUNODOMINANT CD4 VIRAL EPITOPE**

15 **6.1. MATERIALS AND METHODS**

Genetic construction of DEF chimeric molecule. The genes encoding for the I-E^dβ and Ig-Fcγ2a chain fragments were obtained by reverse transcription - polymerase chain reaction ("RT-PCR") from total RNA isolated from the 2PK3 B lymphoma cell line (obtained from the American Type Culture Collection ("ATCC"), accession number ATCC TIB 203) and 14-44-4 hybridoma cells (producing IgG2a, obtained from the ATCC, accession number ATCC HB32), respectively. The gene encoding the I-E^dα chain fragment was obtained from the I-E^dα gene by PCR.

25 The oligonucleotides IE^dβ-RT and Fc-RT were used for retrotranscription of IE^dβ and IgG2a mRNA, respectively. Pairs IE^dβ1-F and I-E^dβ1-R were used for PCR amplification of the leader and the first four codons of the β1 domain of the I-E^d β gene (amino acids -26 through +4). The sequence encoding the eleven amino acids of the HA110-120 peptide and five amino acids of peptide linker containing a BamHI site was introduced into the 3' end of the oligonucleotide IE^dβ-R. Pairs IE^dβ2-F and IE^dβ2-R were used for amplification of codons 5-190 of the IE^dβ gene. Sequence encoding for twelve amino acids of peptide linker containing a BamHI site was included at the 5' end of

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IE^dβ2-F. The sequence encoding the amino acids 228-232 of Fcγ2a-hinge region (containing a natural ApaI site) was included at the 3' end of the IE^dβ2-R. Pairs Fc-F and Fc-R were used for amplification of the sequence encoding the secreted hinge, CH2 and CH3 of the IgG2a gene (amino acids 228-478).

5 A single gene made of the three amplified gene fragments was obtained by digestion with restriction enzymes at sites included in the primers used for amplification and cloned under the polyhedrin promoter of the p2Bac vector (obtained from Invitrogen) at the EcoRI and HindIII sites of the multiple cloning site (FIGURE 1 and FIGURE 2). The p2Bac vector was previously modified at the multiple cloning site
10 by elimination of the nucleotides 2764-2791 containing an ATG codon. The new multiple cloning site became BamHI-EcoRI-SmaI-HindIII.

 Pairs IE^dα-F and IE^dα-R were used for amplification of the sequence encoding the leader, α1 and α2 regions of the IE^dα chain. Sequence encoding a StuI site was included at the 5' end of IE^dα-F and sequence encoding a stop codon and a SpeI site
15 was included at the 3' end of IE^dα-R. The amplified gene was cloned under the p10 promoter of p2Bac in the StuI, SpeI sites. Sequencing of the cloned genes showed in-frame assembling of the genes and the absence of mutations.

Protein production and purification. The baculovirus/insect cell system was used to generate the HA110-120/I-E^dαβ/Fcγ2a dimeric molecule. Recombinant
20 baculoviruses were obtained by cotransfection of linear AcMNPV DNA and DEF-p2Bac vector in SF9 insect cells following manufacturer's instructions (the manufacturer being Invitrogen). Screening and titration of recombinant viruses were done by detection of protein production. Briefly, cell culture supernatants were incubated in 96-well microtiter plates coated with 5 μg/ml goat anti-murine γ2a antibodies and bound DEF molecules
25 were revealed with ¹²⁵I-goat anti-γ2a antibodies. Scale production was obtained by infection of SF9 cells with 10 p.f.u./cell of recombinant baculovirus. Supernatants were monitored daily for protein secretion by a sandwich radioimmunoassay (RIA). The maximum secretion (4 to 5 mg/L) was obtained 4 to 5 days post-infection.

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To prepare DEF protein, the cell culture supernatant was adjusted at pH 7.5, treated with a standard cocktail of protease inhibitors (Boehringer Mannheim) and passed through a goat anti- γ 2a antibody - SepharoseTM affinity column. The eluted material was collected in Tris 1M, pH 8, dialyzed against PBS, and concentrated on CarbowaxTM 20,000 Da (Sigma Chemicals) in dialysis bags of 1,000 dalton MWCO (SpectraporTM). The eventual aggregated material was removed by ultracentrifugation and the protein concentration was estimated by RIA, using a standard curve developed using murine 14-4-4 monoclonal antibody by a previously established protocol (Brumeanu et al., 1996, Immunotechnol. 2:85-95).

Chromatographic characterization. To analyze the molecular size of the affinity-purified DEF molecule, a SuperoseTM 6 column (30 x 1 cm, Pharmacia, LKB) was first equilibrated with ammonium bicarbonate 0.1M, pH 8.0, and then a mixture of MW standard proteins including mouse IgG, chicken egg ovalbumin and cytochrome c was applied to the column at a 1 ml/min flow rate. After standardization, 100 μ g DEF in 200 μ l PBS was applied to the column under identical running conditions and the elution time of the peak-tubes was plotted against the elution time of the MW standard proteins. Individual tubes were also analyzed for the presence of DEF molecule by SDS-PAGE and Western blot.

To analyze the degree of glycosylation by SF9 insect cells, affinity purified DEF was chromatographed on a MONO Q anion exchange column (5/30 HR, Pharmacia, LKB). The column was equilibrated with 20 mM Tris/HCl, pH 7.5, and 100 μ g DEF in 200 μ l equilibrating buffer was applied at a 1 ml/min flow rate over 30 minutes using a 0 to 0.5M NaCl gradient. Tubes were collected at one minute intervals and the peak-tubes were pooled together to be analyzed for the presence of DEF molecule by SDS-PAGE and Western blot.

SDS-PAGE and Western blot analysis. SDS-PAGE was performed in 4-12% gradient gels (Bio-Rad) according to the manufacturer's instruction. Briefly, 5 μ g of the affinity-purified DEF molecule was incubated either at room temperature or boiled in Tris/HCl/SDS 0.1% buffer for 5 min, in the presence or absence of reducing agent (β -

- 16 -

mercaptoethanol, 2-ME). Samples were electrophoresed for 45 minutes at 150 volts, and the gels were either silver-stained or electrotransferred in semidry conditions for 45 minutes at 200 mAmps/gel onto PVDF membranes (0.2 μ). Membranes were blocked overnight at 4°C with 5% fat-free milk (Carnation) in PBS, incubated for 2 hours at room temperature with 125 I-14-4-4 monoclonal antibody in PBS/BSA 1% at 10^7 cpm/membrane, then washed extensively with 0.05% TweenTM 20 in PBS, and exposed overnight on KodakTM X-OMAT film at -80°C.

FACS analysis. Purified transgenic T cells (1×10^6), expressing the 14.3d TCR specific for I-E^d class II/HA110-120 peptide complex (Kirberg et al., 1994, J. Exp. Med. 180:25-34), or 14-3-1 TcH in 100 μ l PBS/BSA 1 % were incubated for 30 minutes on ice with 10 μ g/ml purified DEF in the presence or in the absence of 100 μ g/ml 6.5.2 anti-TCR clonotypic monoclonal antibody (Weber et al., 1992, Nature 356:793-796). Cells were washed in cold PBS/BSA 1%/NaN₃ 0.1% and bound DEF molecules were labeled with goat anti- γ 2a monoclonal antibody FITC conjugate (Boehringer Mannheim) for 30 minutes on ice. As a control of self-fluorescence cells were also stained with goat anti- γ 2a FITC conjugate. The percentage of transgenic T cells expressing the specific TCR was estimated by staining with 6.5.2 anti-TCR clonotypic antibody conjugated to FITC.

B_{Long} melanoma cells expressing a murine Fc γ RII (Weinshank et al., 1988, J. Exp. Med. 167:1909-1925), were incubated with 10 μ g/ml purified DEF or 14-4-4 mAb as a control (mouse IgG2a, (ATCC HB32)), in the presence or in the absence of the 2.4G-2 anti-Fc γ R mAb (Unkeless, 1979, J. Exp. Med. 150:580-596) and labeled with goat anti- γ 2a-FITC. The percentage of positive cells was scored among 5,000 cells.

Complement-mediated cell cytotoxicity. Transgenic T cells were purified from the spleens of transgenic mice on Ficoll-Hypaque followed by enrichment on nylon-wool columns as described in Brumeanu et al., 1996, Nature Biotechnology 14:722-725. Purified T cells were incubated for 30 minutes at room temperature with various concentrations of DEF or 14-4-4 mAb as control (2 μ g-50 ng). Rabbit complement (Sigma Chemicals) was added for 45 minutes and then the cells were stained

- 17 -

with eosin and fixed with formaldehyde according to a standard protocol (Thompson, R.A. (ed.), 1981, *Techniques in Clinical Immunology*, Blackwell Scientific Publications, Oxford). The percentage of lysed cells was determined microscopically in an inverted Zeiss microscope.

5

6.2. RESULTS

Structural integrity of the DEF molecule. As illustrated in FIGURE 3A, the affinity-purified DEF molecules eluted from the Superose-6 column as a molecule of 170 kDa. This size indicated that the soluble DEF molecule was secreted by the SF9 infected cells as a homogeneous dimer. A minor degree of heterogeneity due to different glycosylation by insect cells was revealed by anion exchange chromatography (FIGURE 3B).

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SDS-PAGE under non-reducing/non-boiled conditions revealed a single band of 170 kDa (FIGURE 4A, lane 2). The 170 kDa band corresponds to the molecular size of an intact HA110-120/I-E^dαβ/Fcγ2a molecule, in agreement with the result obtained by size exclusion chromatography. The major bands of 110 and 30 kDa obtained after boiling the DEF molecule in the presence of detergent correspond to the molecular size, respectively, of HA110-120/I-E^dβ/Fcγ2a dimer and single chain I-E^dα. The minor bands of 55 and 80 kDa correspond to the molecular size of the HA110-120/I-E^dβ/Fcγ2a monomer and HA110-120/I-E^dαβ/Fcγ2a dimer, respectively.

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These monomers were generated under boiling conditions, by dissociation of the dimeric DEF molecule. Under reducing and non-boiling conditions, the DEF molecule migrated as three major bands of 50, 55 and 80 kDa which correspond to HA110-120/I-E^dβ/Fcγ2a and HA110-120/I-E^dαβ/Fcγ2a monomers respectively, and a minor band of 30 kDa which corresponds to I-Eα chain (FIGURE 4A, lane 3). Under reducing and boiling conditions, DEF molecule migrated as two bands of 50 and 55 kDa as well as 30 kDa, which correspond to the HA110-120/I-E^dβ/Fcγ2a monomer and I-Eα chain, respectively. The two bands of 50 and 55 kDa of the HA110-120/I-E^dβ/Fcγ2a (FIGURE 4A, lanes 3 and 4) may represent two major forms of glycosylation as also found by anion exchange chromatography. Western blot analysis developed with goat anti-mouse γ2a antibodies

- 18 -

revealed also the 170 kDa band under nonreducing and no boiling conditions, and two bands of 50 and 55 kDa under reducing and boiling conditions. This indicates that the dimeric DEF was correctly folded and preserved the antigenicity of the Fc γ 2a fragment (FIGURE 4B, lanes 1 and 2). Dimeric DEF molecule was also revealed under

5 nonreducing and no boiling conditions by 14-4-4 monoclonal antibody which recognizes a conformational epitope of I-E^d α (Ozato et al., 1980, J. Immunol. 124:533-540; FIGURE 4B, lane 3), but not under reducing and boiling conditions (FIGURE 4B, lane 4). Similar results on the antigenicity of DEF molecule were obtained in RIA using plates coated with goat anti- γ 2a antibodies and revealed with either ¹²⁵I-goat anti- γ 2a antibodies or ¹²⁵I-

10 14-4-4 mAb.

Binding of DEF molecule to HA110-120-specific TCR. We determined the ability of HA110-120/I-E^d complex of DEF chimeric molecule to bind to HA110-120 specific TCR. For this, we used either 14-3-1 T cell hybridoma (TcH) or purified transgenic T cells expressing the 14.3d TCR which recognizes the HA110-120/I-E^d

15 complex. TcH were incubated with DEF molecules and then labeled with goat anti- γ 2a-FITC. FACS analysis showed that 68% of T cells bound DEF (FIGURES 5B and 5A, respectively). Similar results were obtained by staining with the 6.5.2 mAb specific for the 14.3d TCR. The binding of DEF to TCR was inhibited by 6.5.2 mAb (FIGURE 5C). When T cells purified from transgenic mice were stained with DEF molecules, we

20 detected 32% positive T cells. The same percentage was obtained by staining the purified transgenic T cells with 6.5.2 mAb-FITC conjugate.

Binding of DEF molecule to Fc γ R. To investigate the binding ability of the Fc γ 2a fragment expressed by DEF molecule to the Fc γ R, we used B_{long} melanoma cell line cells transfected with the Fc γ RII receptor gene. Cells were incubated with either

25 DEF molecules, or 14-4-4 mAb as positive control, and stained with goat anti- γ 2a-FITC conjugate. Data depicted in FIGURES 5E and 5H, respectively, show the binding of DEF molecule and 14-4-4 mAb to B_{long} cells as compared to the self-fluorescence control (FIGURE 5B and 5C). The binding of both DEF molecule and 14-4-4 mAb was inhibited by 2.4G-2 rat anti-Fc γ R mAb (FIGURES 5F and 5I, respectively).

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Complement-mediated cytotoxicity. The activation of the complement cascade by IgG2a depends mainly on the structural integrity of the C1q binding site located on the hinge region and the CH2-CH3 domains of the Fc fragment, particularly on the residues Glu 318, Lys 320, Lys 322 and Pro 331 (Reid et al., 1995, The Immunologist 3:206-211). To determine whether the folding of the Fcγ2a domains was preserved within the DEF molecule, we measured the complement-mediated lysis induced by DEF on T cells from transgenic mice. Cells stained with eosin and visualized microscopically revealed approximately 30% lysis. According to FACS analysis, a similar percentage (32%) of HA110-120-specific T cells, as found in the preparation of purified transgenic cells used in this assay, was stained by DEF molecule or 6.5.2 mAb.

6.3. DISCUSSION

We have generated a disulfide-stabilized dimer of the CD4 T cell immunodominant epitope of HA of the A/PR/8/34 influenza virus, covalently linked to a murine I-E^d-Ig chimeric molecule (DEF). Nucleotide sequencing of the chimeric genes indicated that the gene fragments were assembled in frame, and that no mutations occurred during the process of genetic construction. The recombinant DEF molecule was secreted by the transfected SF9 insect cells as a 170 kDa soluble dimer. The dimer was stable in the presence of detergent (0.1% SDS), which indicated that the HA110-120 peptide was correctly assembled in the groove of the DEF molecule, being able to stabilize the MHC heterodimer. Indeed, it was previously shown that only the I-E^d class II molecules loaded *in vitro* with peptide, but not the empty class II molecules, migrate in SDS-PAGE under nonreducing/no boiling conditions as a single protein component (Germain et al., 1996, Immunol. Rev. 151:5-30).

The recombinant DEF dimer preserved the antigenicity of both MHC and Fcγ2a fragments. This indicated that both extracellular domains of the I-E^dα and I-E^dβ chains, as well as the Fcγ2a regions of IgG2a, were assembled and folded correctly. As a result, the MHC/peptide portion of DEF molecule bound to a peptide-specific TCR expressed either on a TcH or on transgenic T cells.

- 20 -

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

10

(i) APPLICANT: Casares, Sofia
Brumeanu, Teodor Doru
Bona, Constantin

15

(ii) TITLE OF THE INVENTION: EPITOPE-BEARING MAJOR HISTOCOMPATIBILITY
COMPLEX CLASS II ELEMENT/IMMUNOGLOBULIN CHIMERIC MOLECULES

20

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Baker & Botts, LLP
(B) 30 Rockefeller Plaza
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(D) STATE: NY
(E) COUNTRY: US
(F) ZIP: 10112

25

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FASTSEQ Version 1.5

30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION

35

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:

40

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Kole, Lisa B
(B) REGISTRATION NUMBER: 35,225

45

(iv) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 212-408-2500
(B) TELEFAX: 212-765-2519
(C) TELEX:

50

(2) INFORMATION FOR SEQ ID NO:1

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 21 -

(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

5

(x) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATTCATC

9

10

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 186 base pairs
(B) TYPE: nucleic acid I-E^d β HA 110-120 and peptide linker
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

25

(x) SEQUENCE DESCRIPTION: SEQ IS NO:2:

TGCAGCATGG TGTGGCTCCC CAGAGTTCCC TGTGTGGCAG CTGTGATCCT	50
GTTGCTGACA GTGCTGAGCC CTCCAGTGGC TTTGGTCAGA GACACCTCTT	100
TTGAAAGATT TGAGATCTTC CCAAAGGAAG GAGGTGGTGG ATCCGGTGGA	150
GGGGGAAGTG GAGGTGGAGG GTCTAGACCA CGGTTT	186

30

(4) INFORMATION FOR SEQ ID NO:3

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid I-E^d, HINGE
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

45

(x) SEQUENCE DESCRIPTION: SEQ IS NO:3:

50

GAGTGGAAAG AGCCCAGAGG GCCCACA
27

55

(5) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

- 22 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:4:

AAATGCCCAG CACCTAAC

18

(6) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:5:

AAACCCAAAG GGTCAGTA

18

(6) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:6:

AAATGAAAGC TT

12

(7) INFORMATION FOR SEQ ID NO 7

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 60 amino acids

(B) TYPE: peptide

- 23 -

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:7:

Met Val Trp Leu Pro Arg Val Pro Cys Val Ala Ala Val Ile Leu Leu Leu Thr Val Leu Ser Pro Pro Val Ala
Leu Val Arg Asp Thr Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys Glu Gly Gly Gly Gly Ser Gly Gly Gly Gly
Ser Gly Gly Gly
Gly Ser Arg Pro Arg Phe

(8) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 9 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:8:

Glu Trp Lys Glu Pro Arg Gly Pro Thr

9

(9) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:9:

Lys Cys Pro Ala Pro Asn

6

(10) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: peptide

- 24 -

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(x) SEQUENCE DESCRIPTION: SEQ IS NO:10:

5

10

Lys Pro Lys Gly Ser Val

6

We Claim:

1. An immunologically active molecule comprising four protein chains forming two heterodimers, wherein each heterodimer comprises (i) a first protein chain comprising an immunoglobulin constant region component linked to a MHC class II α or β chain component and (ii) a second protein chain comprising the complementary MHC class II chain component, wherein the first protein chain, the second protein chain, or both protein chains further comprise an epitope of interest, and wherein the two heterodimers are joined together via one or more disulfide bonds between immunoglobulin constant region components.
2. The immunologically active molecule of claim 1, wherein the first protein chain comprises the immunoglobulin constant region component linked to a MHC class II α chain component and the epitope of interest and the second protein chain comprises a MHC class II β chain component.
3. The immunologically active molecule of claim 2, wherein the second protein further comprises the epitope or interest.
4. The immunologically active molecule of claim 1, wherein the first protein chain comprises the immunoglobulin constant region component linked to a MHC class II β chain component and the epitope of interest and the second protein chain comprises a MHC class II α chain component.
5. The immunologically active molecule of claim 4, wherein the second protein further comprises the epitope of interest.
6. The immunologically active molecule of claim 1, wherein the first protein chain comprises the immunoglobulin constant region component linked to a MHC class II α chain component and the second protein chain comprises a MHC class II β chain component and the epitope of interest.

7. The immunologically active molecule of claim 1, wherein the first protein chain comprises the immunoglobulin constant region component linked to a MHC class II β chain component and the second protein chain comprises a MHC class II α chain component and the epitope of interest.
8. The immunologically active molecule of any one of claims 1-7, wherein the MHC class II components are HLA-DR proteins.
9. The immunologically active molecule of claim 8, wherein the HLA-DR is HLA-DR2.
10. The immunologically active molecule of claim 8, wherein the HLA-DR is HLA-DR4.
11. The immunologically active molecule of any one of claims 1-10, wherein the epitope of interest is associated with an autoimmune disease.
12. A use of an effective amount of the immunologically active molecule of any one of claims 1-11 for treating an autoimmune disease.
13. A use of an effective amount of the immunologically active molecule of any one of claims 1-11 for treating a graft versus host disease.
14. A use of an effective amount of the immunologically active molecule of any one of claims 1-11 for amelioration or prevention of graft versus host disease.

1/7

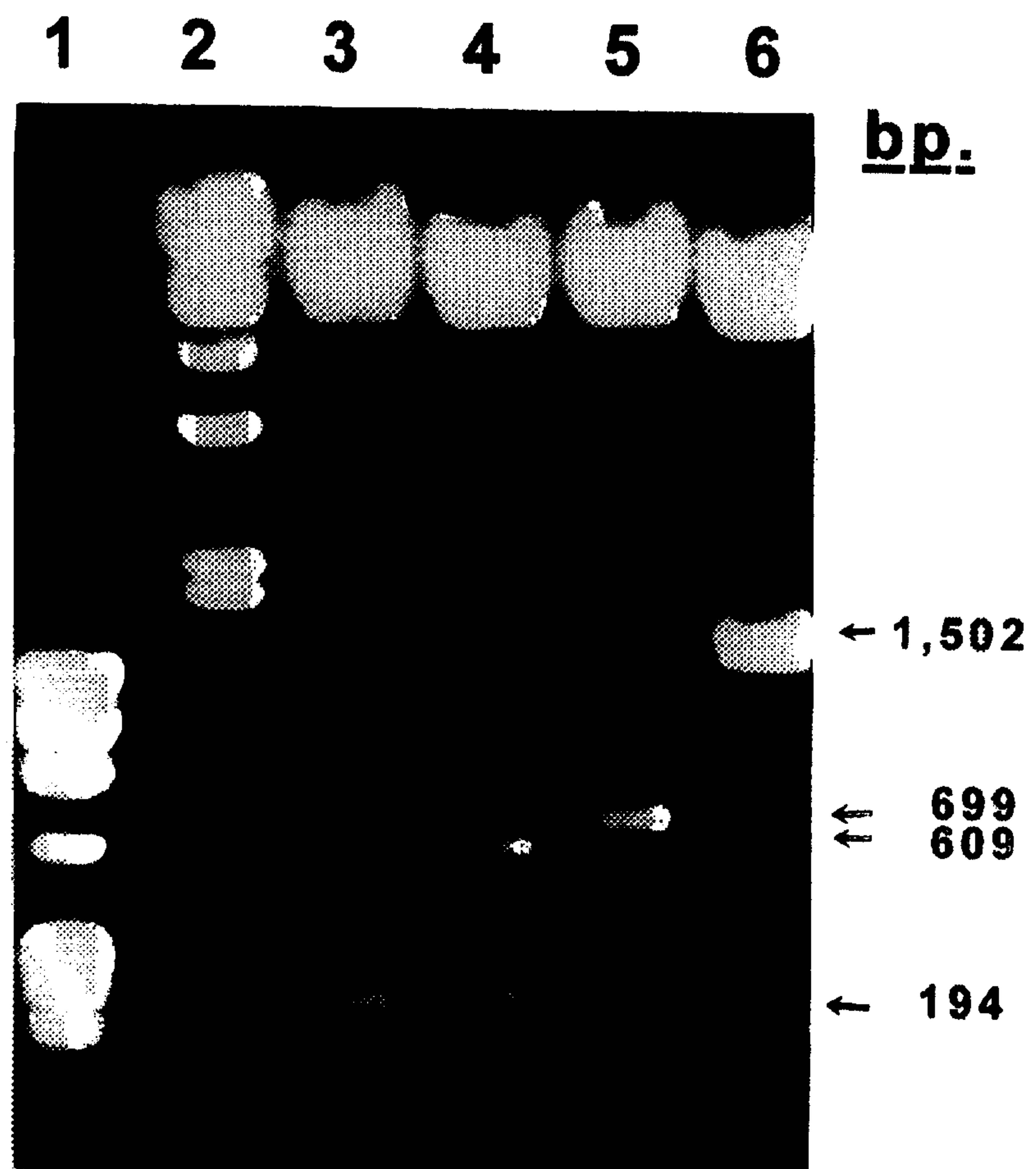


FIG.1

GCATTCATC // TGCAGC ATG GTG TGG CTC CCC AGA GTT CCC TGT GTG GCA GCT GTG ATC CIG TIG CTG ACA
M V W L P R V P C V A A V I L L L T
----- I-E^d SIGNAL PEPTIDE -----
GTG CTG AGC CCT CCA GTG GCT TIG GTG AGA GAC ACC ICT TTT GAA AGA TTT GAG ATC TTC CCA AAG GAA
V L S P P V A L V R D T S F E R F E I F P K E
----- I-E^dβ ----- HA110-120 PEPTIDE -----
GGA GGT GGT GGA ICC GGT GGA GGA AGT GGA GGT GGA GCG TCT AGA CCA CCG TTT // GAG TGG AAA
G G G S G G G G G S G G G S R P R F E W K
----- PEPTIDE LINKER ----- I-E^dβ -----
GAG CCC AGA GGG CCC ACA // AAA TGC CCA GCA CCT AAC // AAA CCC AAA GCG TCA GTA // AAA TGA AAG CTT
E P R G P T K C P A P N K P K G S V K TERM
----- HINGE REGION ----- CH₂ ----- CH₃ -----

FIG.2A

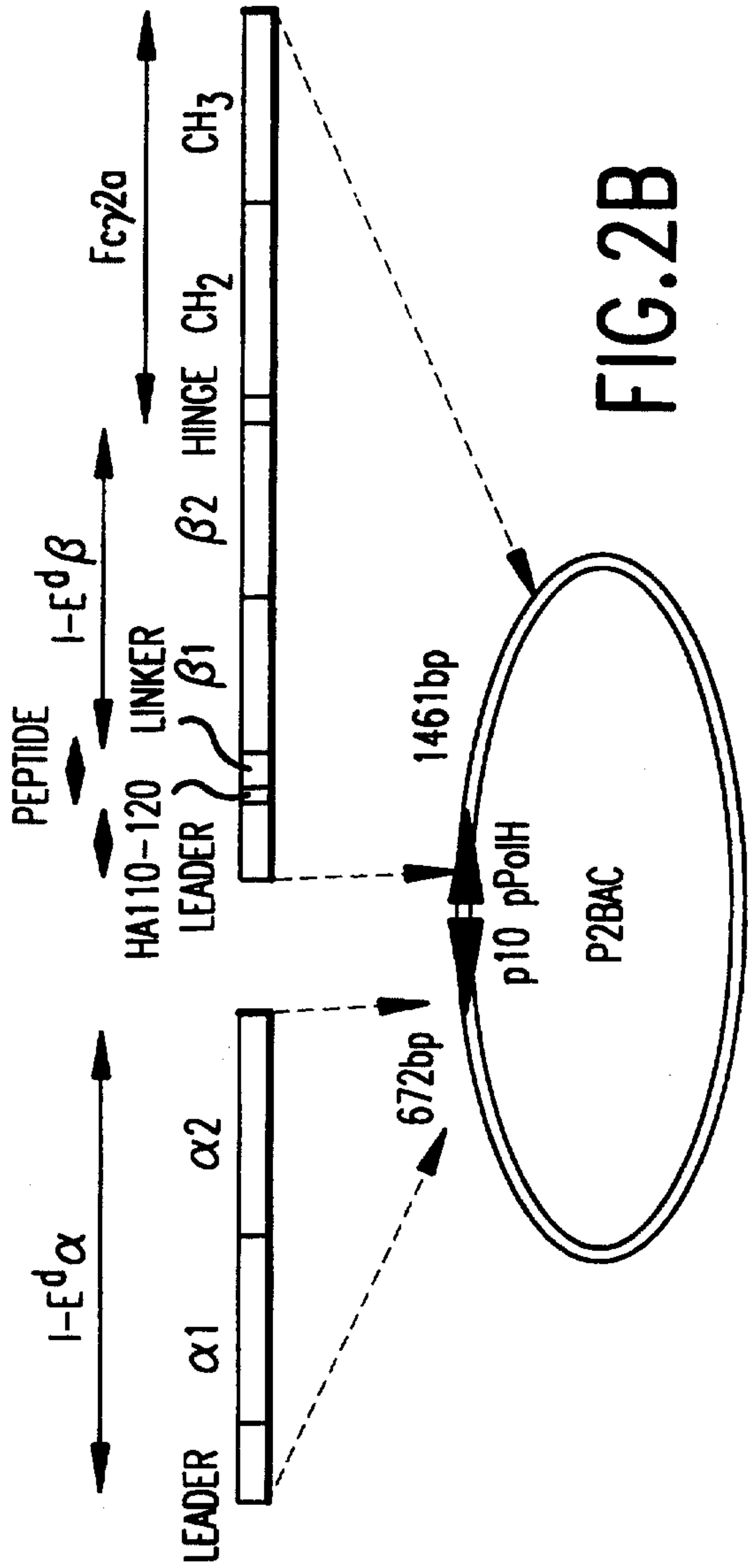


FIG.2B

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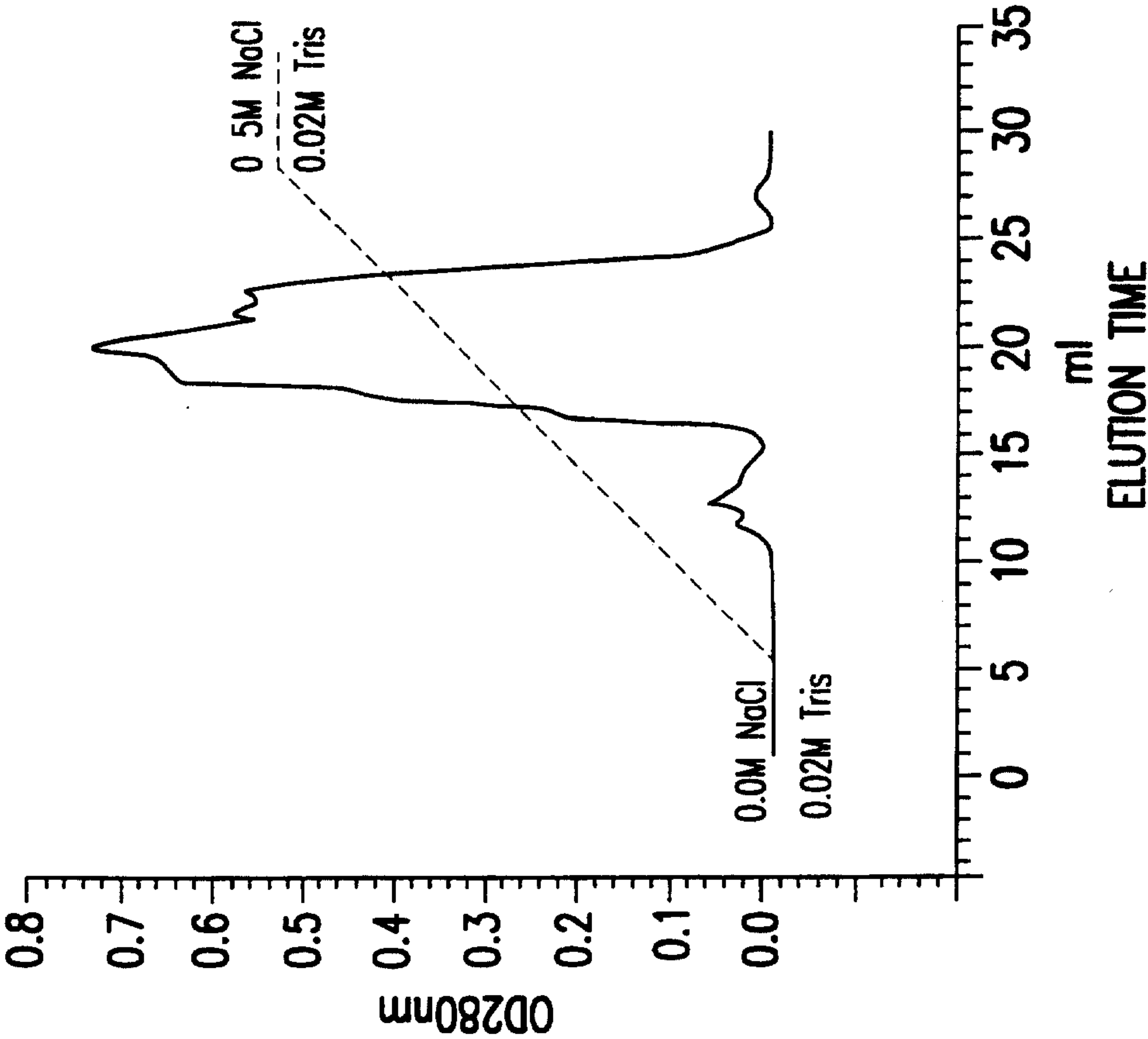


FIG.3B

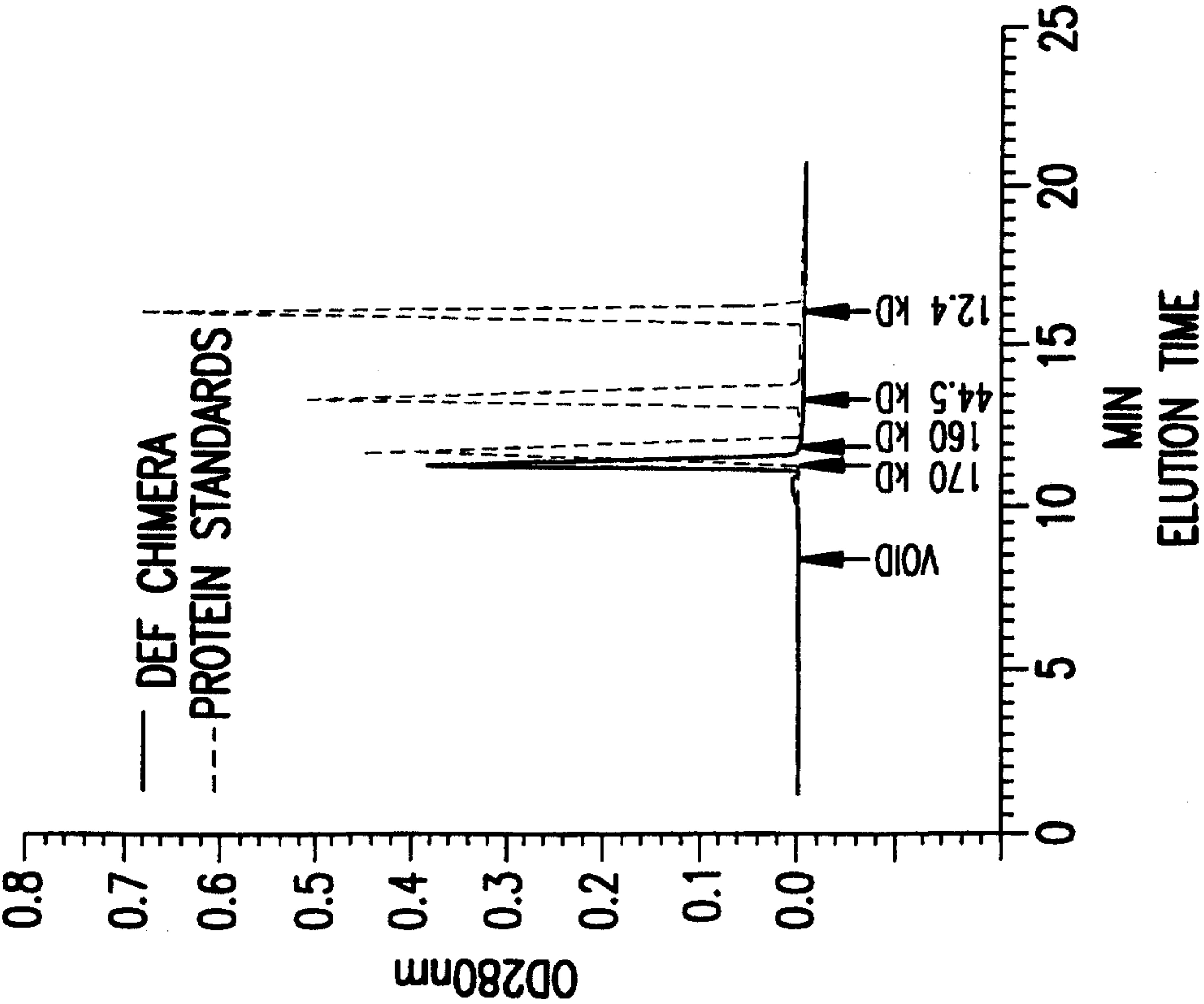


FIG.3A

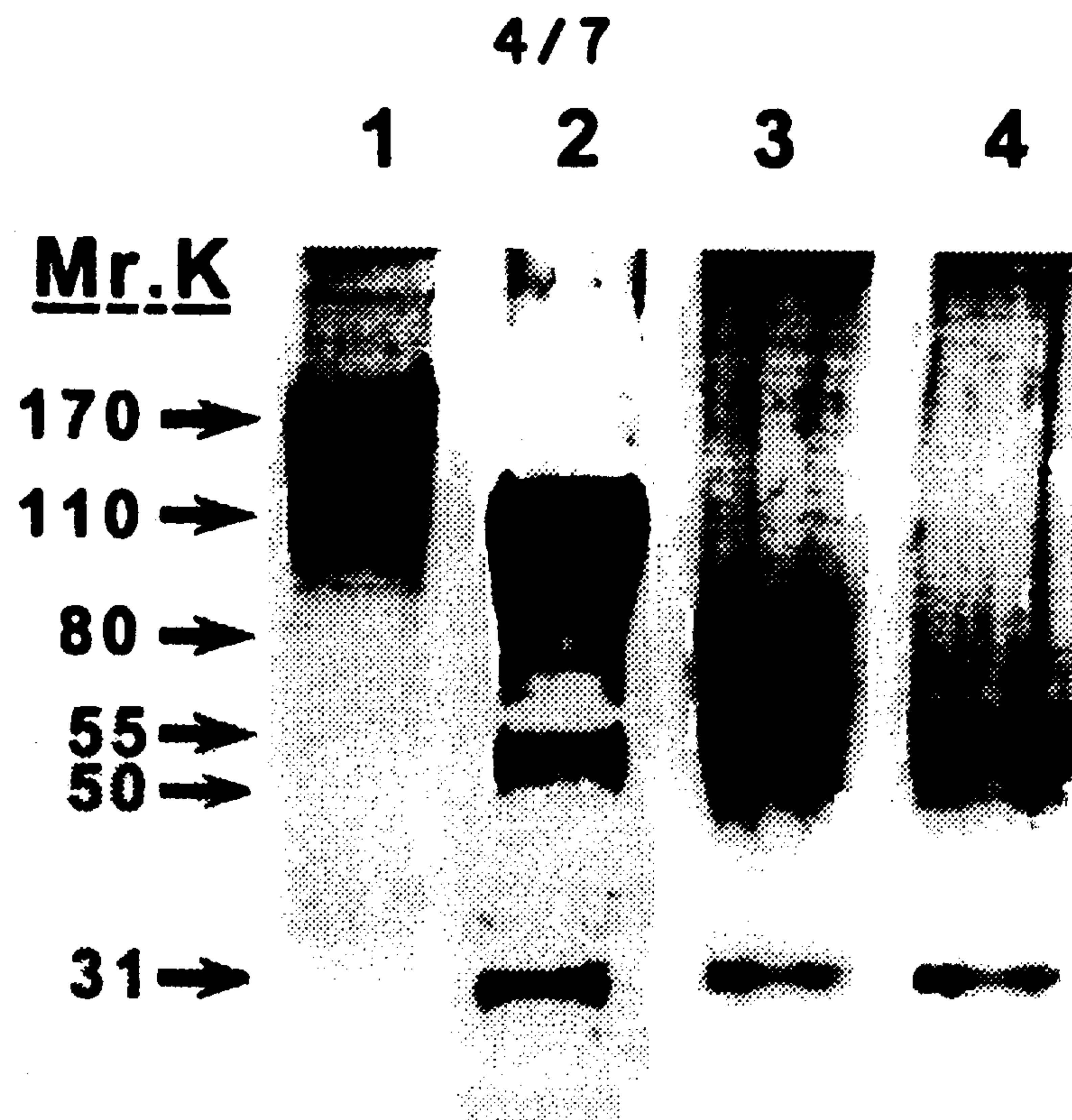


FIG.4A

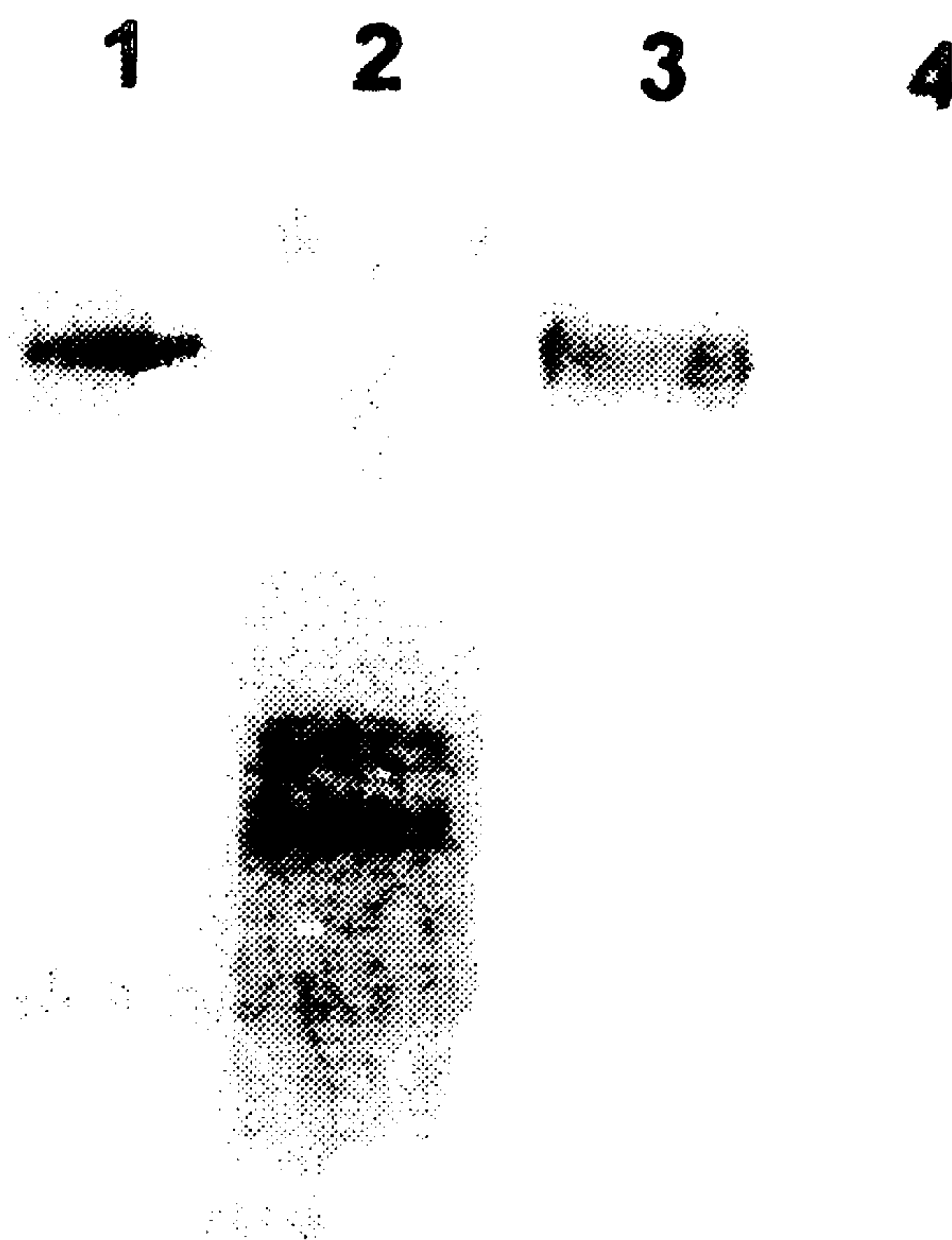


FIG.4B

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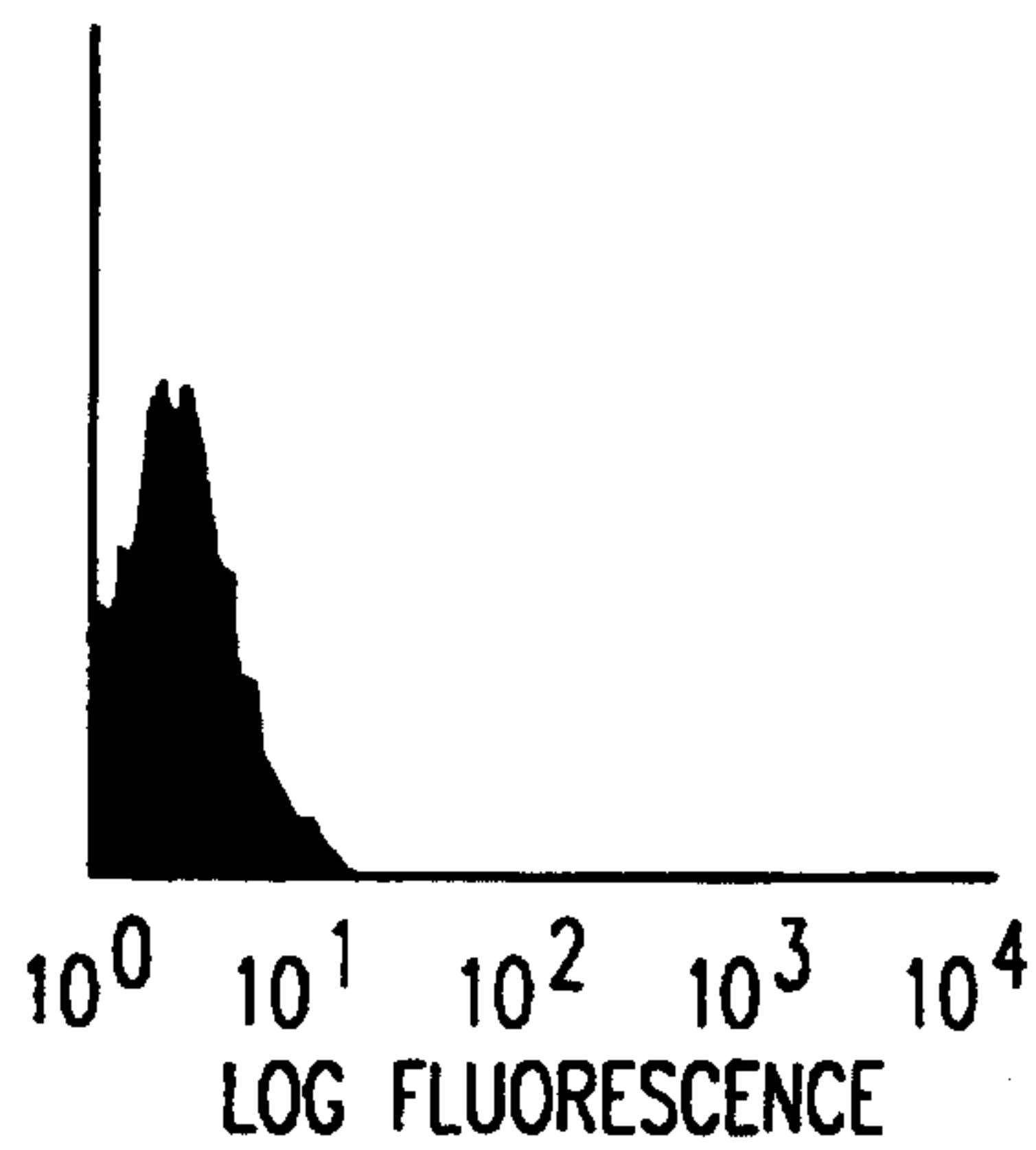


FIG. 5A

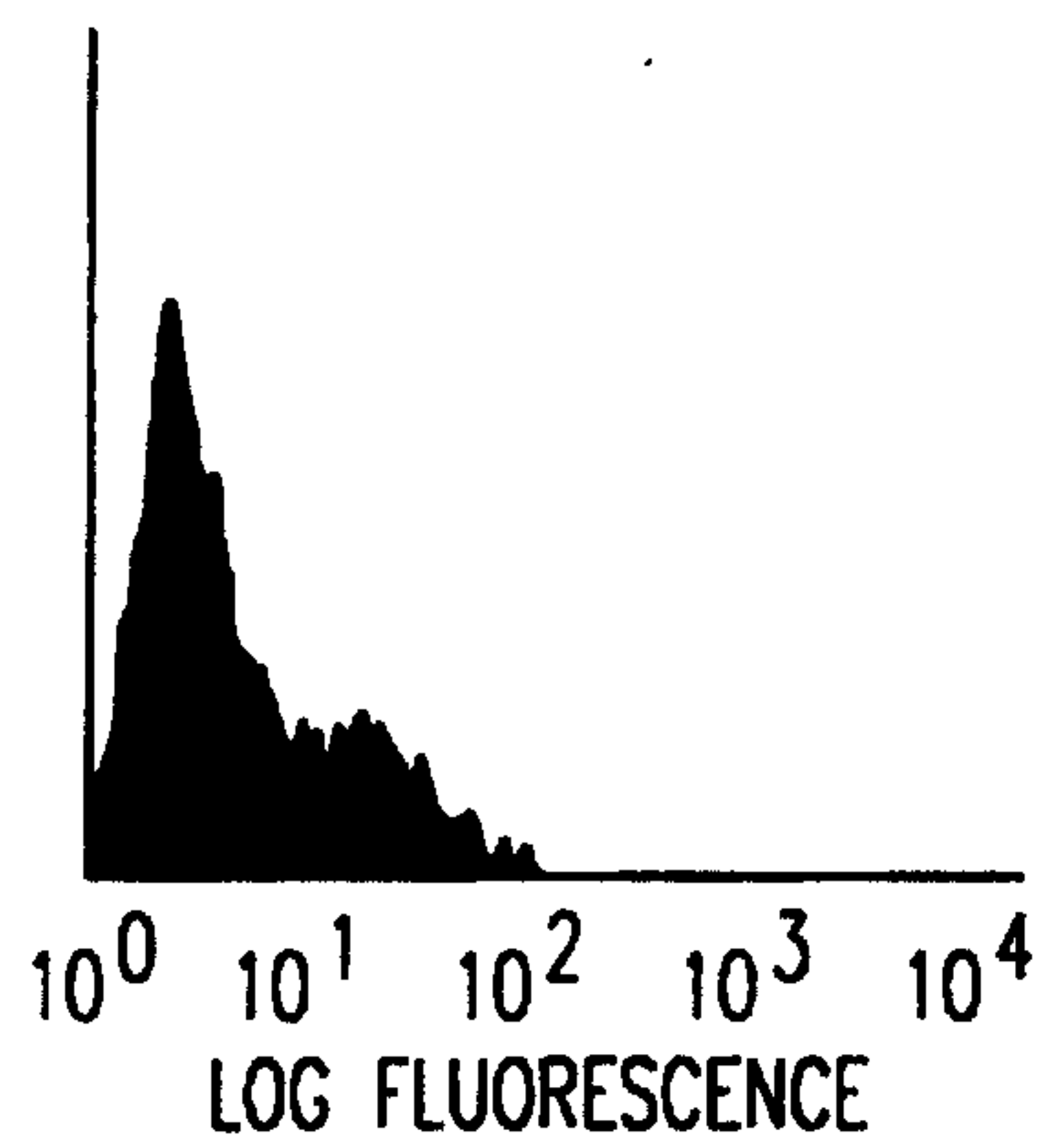


FIG. 5D

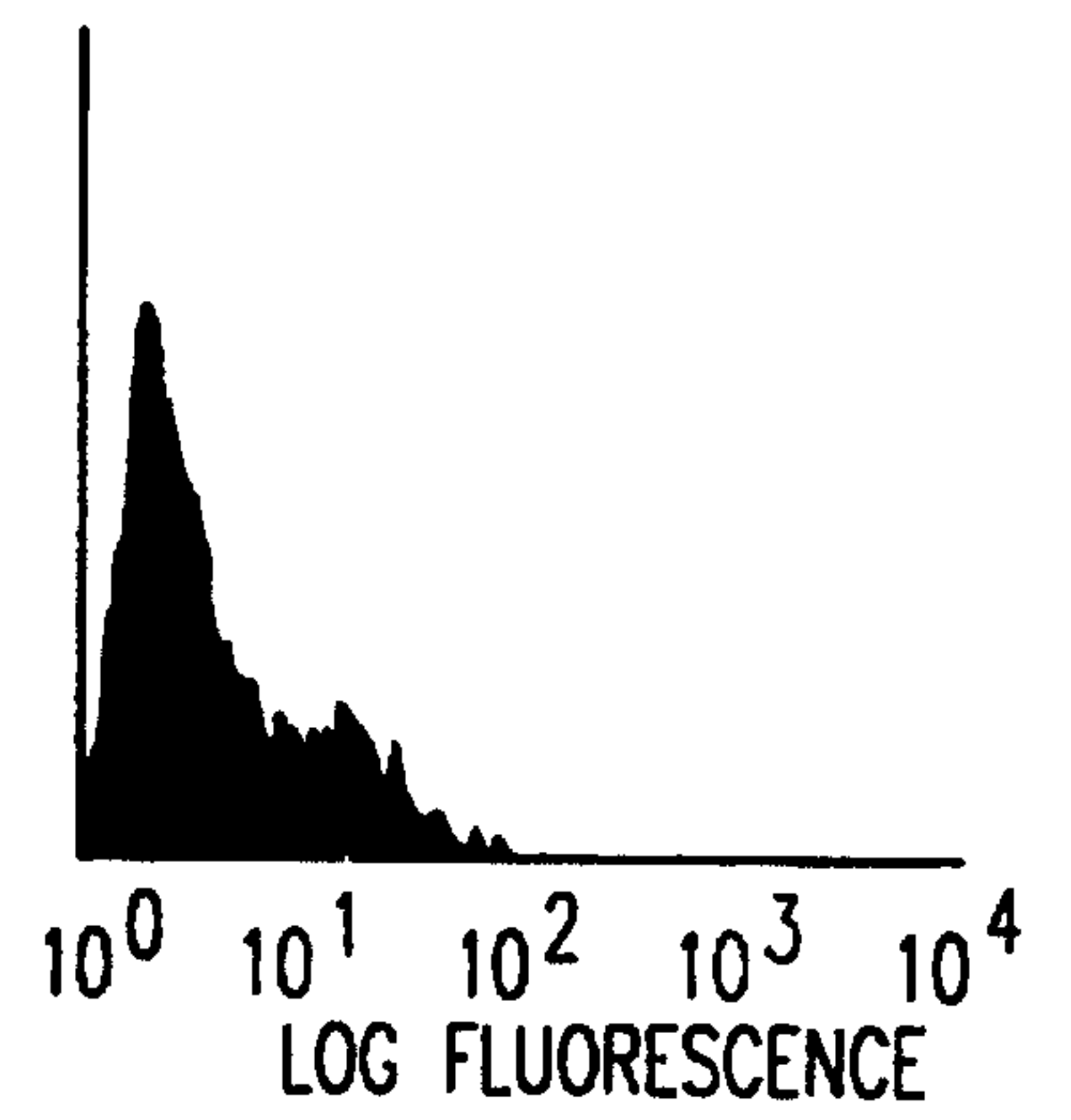


FIG. 5G

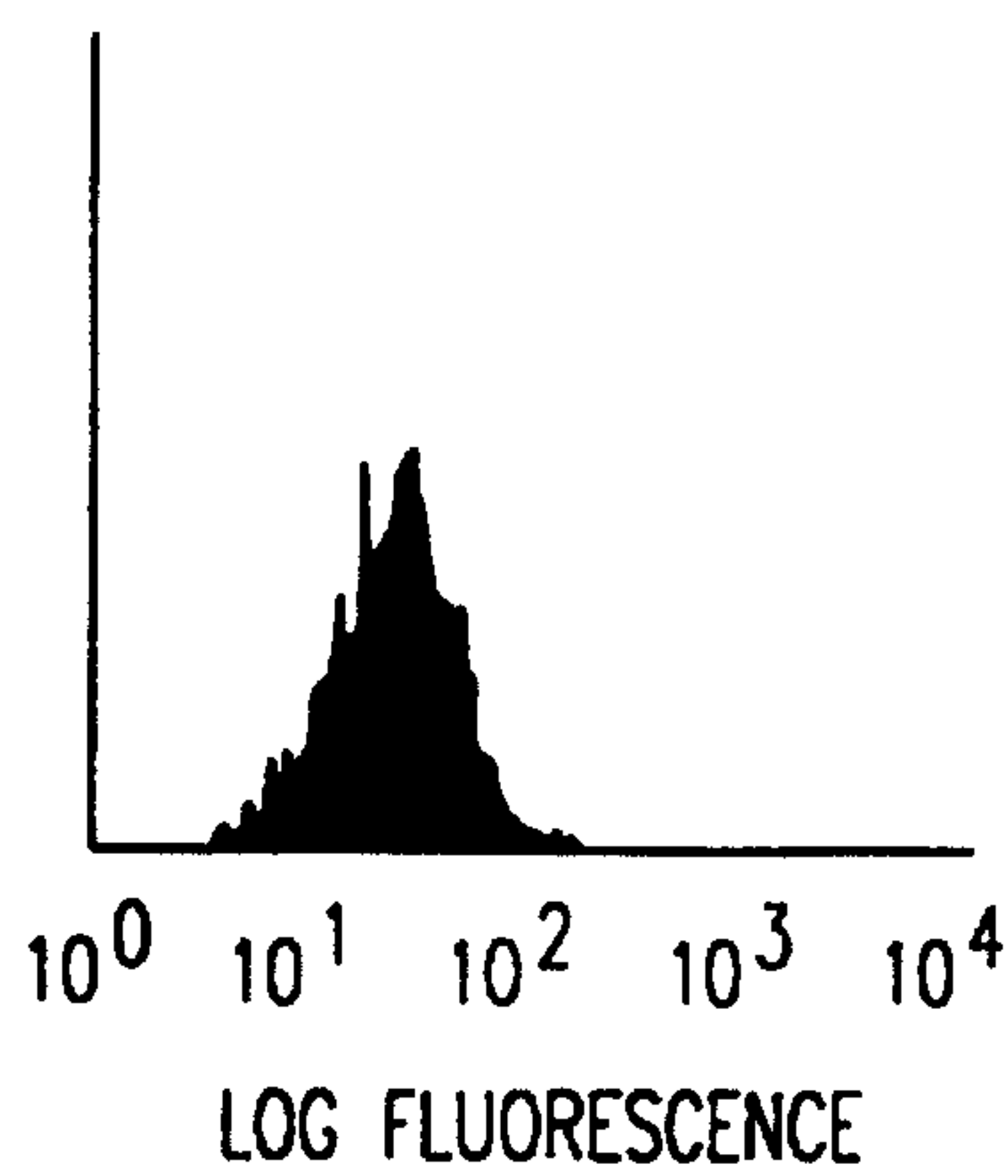


FIG. 5B

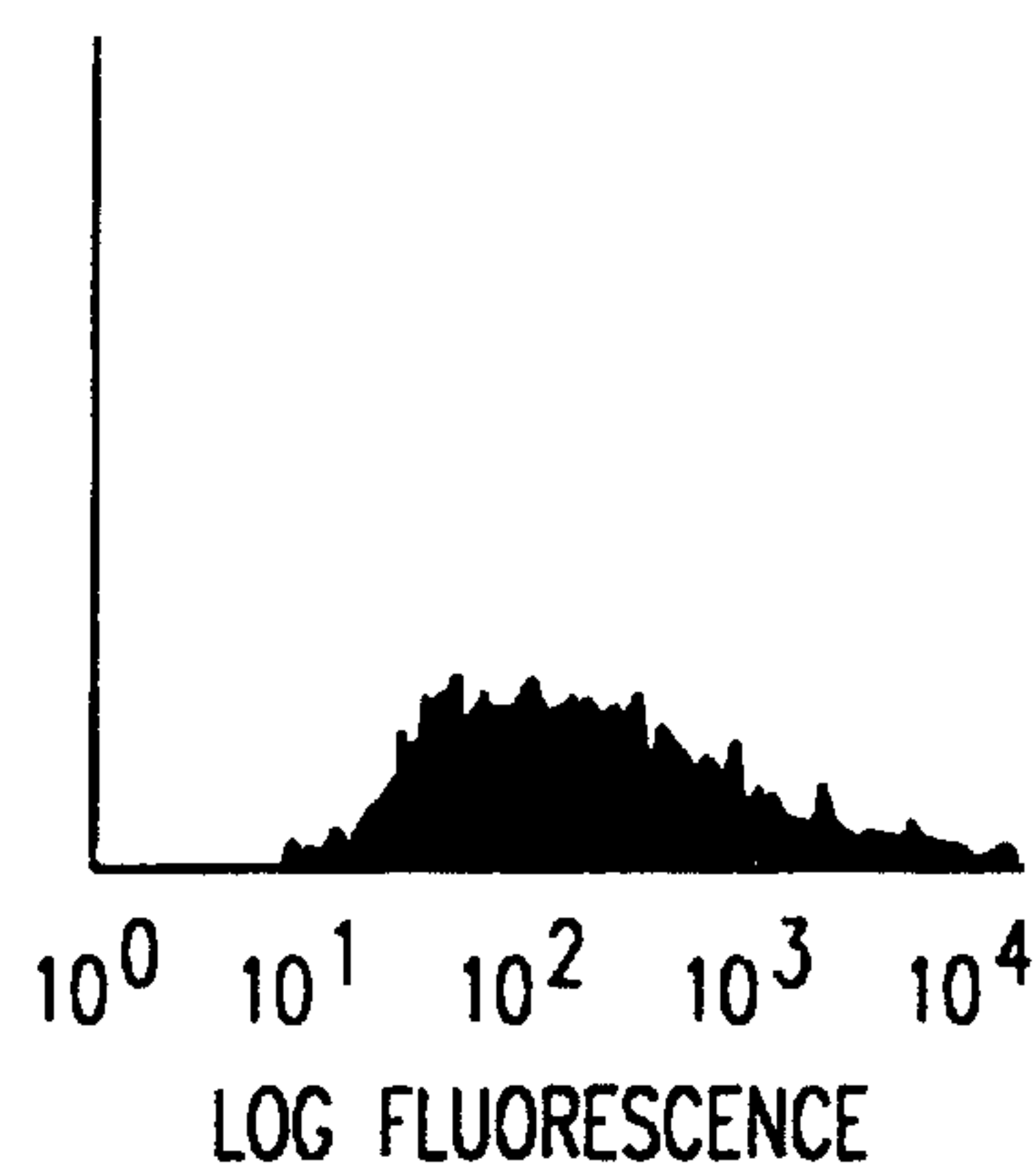


FIG. 5E

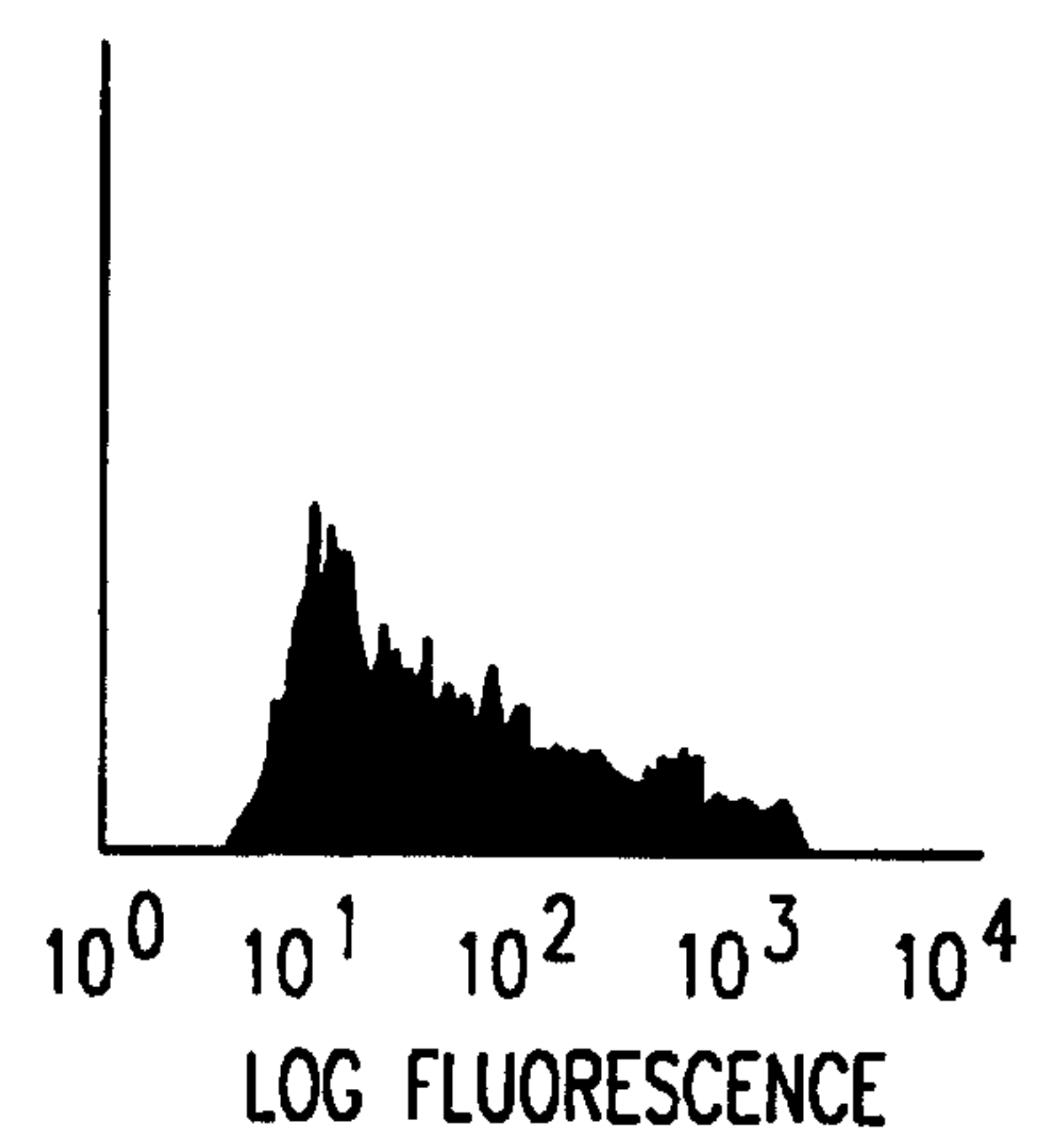


FIG. 5H

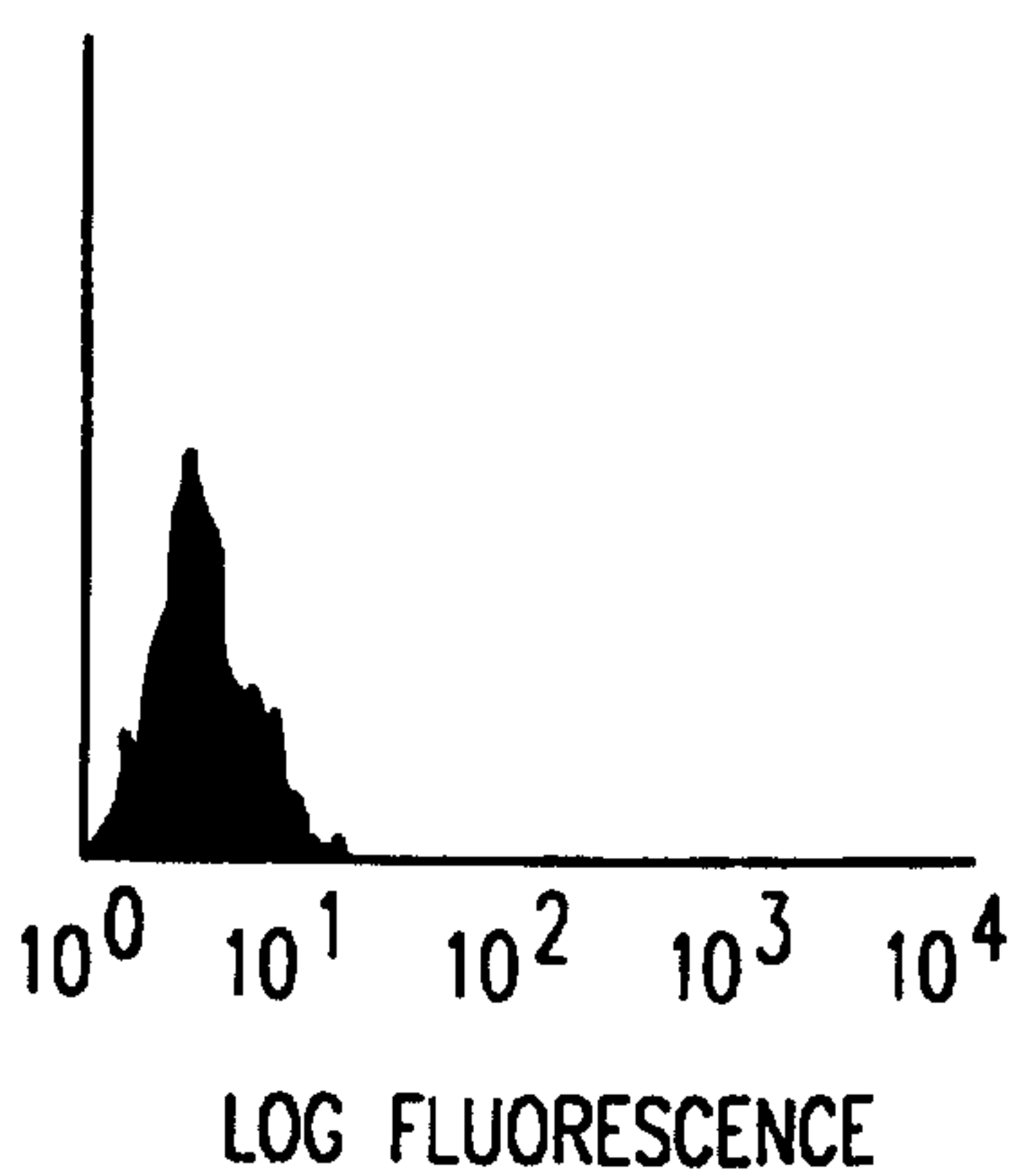


FIG. 5C

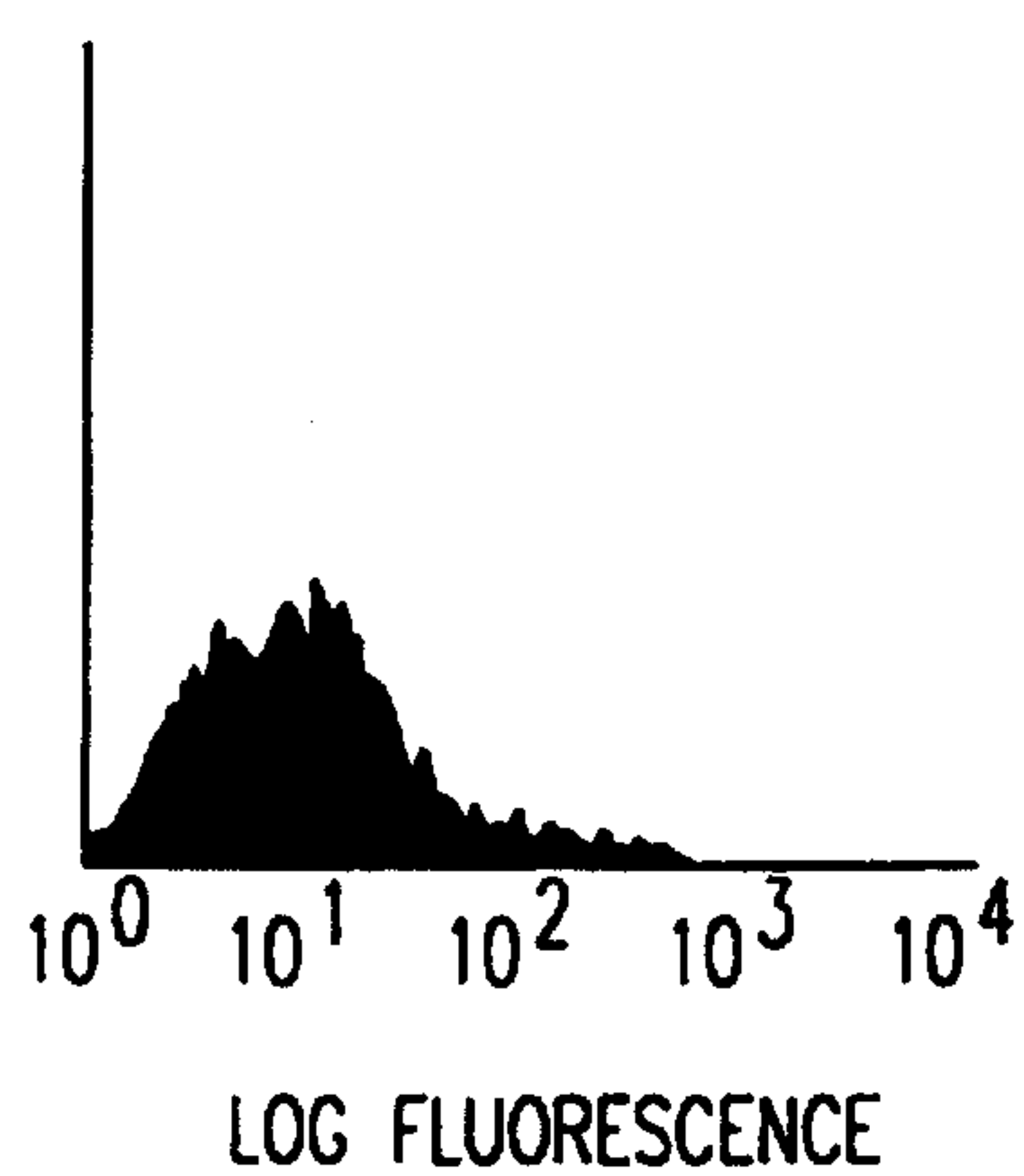


FIG. 5F

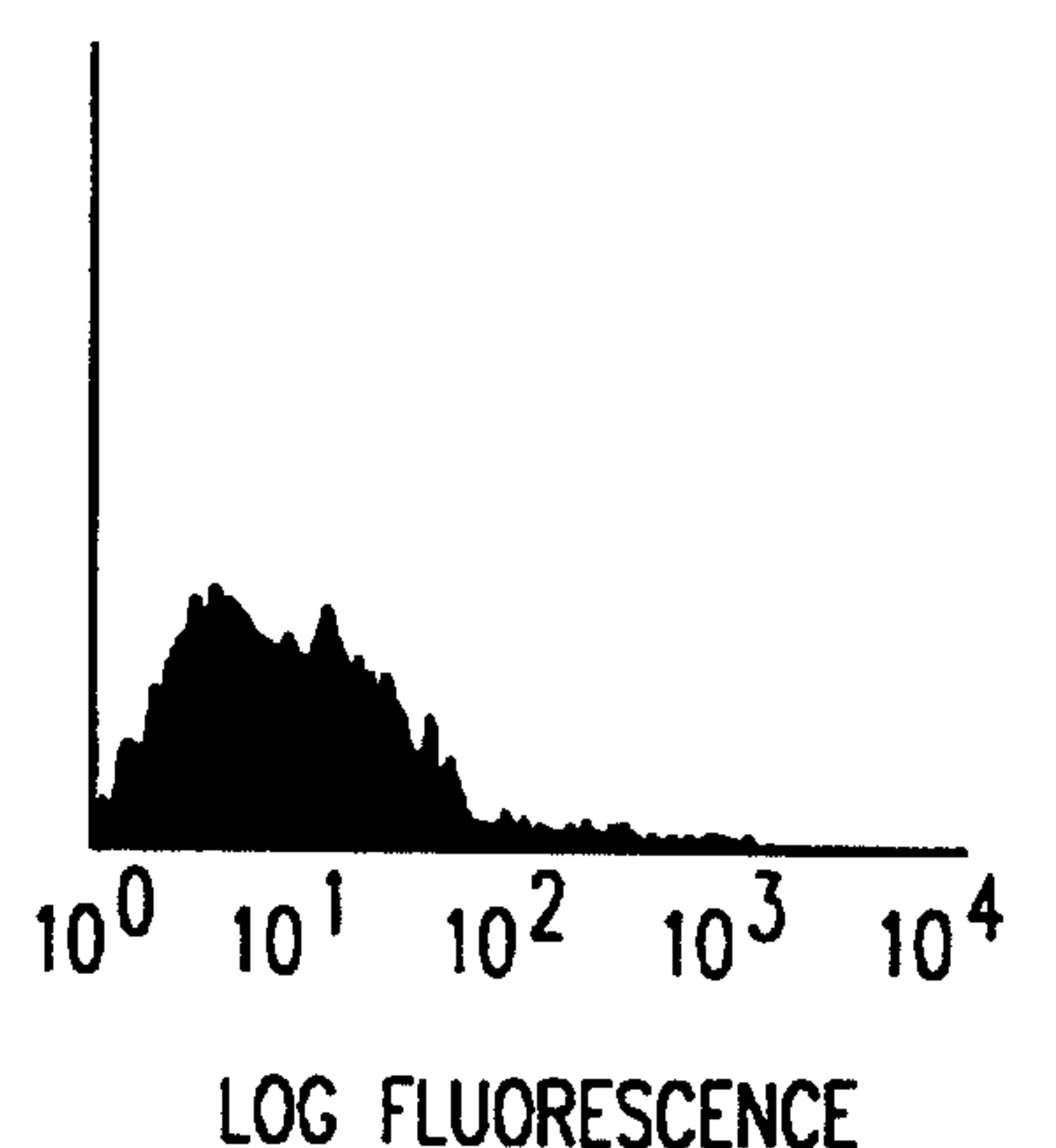


FIG. 5I

COMPLEX	H2										Tla		
MHC CLASS	I	II	III			I	I	I	I	I			
GENETIC REGION	K	I	S			D	Qa	Tla					
GENES (CHROMOSOME 17)	κ	A β A α E β E α	C4	Slp	FB	C2	TNF α	TNF β	D	L	Qa(17)	Tla	Qa(12)
GENE PRODUCTS	H-2K	I-A I-E	C4	C4'	FB	C2	TNF α	TNF β	H-20	H-2L	Qa(2-5)	Tla	Qa1.6

FIG.6

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HLA															
II					III					I					
DPβ DPα	DQβ DQα	DRβ DRα	210HB	C4B	210HA	C4A	FB	C2	HSP1	HSP2	TNFα	TNFβ	B	C	A
HLA-DP	HLA-DQ	HLA-DR	210HB	C4B	210HA	C4A	FB	C2	HSP1	HSP2	TNFα	TNFβ	HLA-B	HLA-C	HLA-A

FIG.7