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(54) Title: ANTITHROMBOTIC AGENTS

(57) Abstract: Monoclonal antibodies as antithrombotic agents.

ANTITHROMBOTIC AGENTS**FIELD OF THE INVENTION**

This invention relates to monoclonal antibodies
5 (mAbs) that bind to a human coagulation factor or
cofactor and their use as inhibitors of thrombosis.

BACKGROUND OF THE INVENTION

Under normal circumstances, an injury, be it minor
10 or major, to vascular endothelial cells lining a blood
vessel triggers a hemostatic response through a sequence
of events commonly referred to as the coagulation
"cascade." The cascade culminates in the conversion of
soluble fibrinogen to insoluble fibrin which, together
15 with platelets, forms a localized clot or thrombus which
prevents extravasation of blood components. Wound
healing can then occur followed by clot dissolution and
restoration of blood vessel integrity and flow.

The events which occur between injury and clot
20 formation are a carefully regulated and linked series of
reactions. In brief, a number of plasma coagulation
proteins in inactive proenzyme forms and cofactors
circulate in the blood. Active enzyme complexes are
assembled at an injury site and are sequentially
25 activated to serine proteases, with each successive
serine protease catalyzing the subsequent proenzyme to
protease activation. This enzymatic cascade results in
each step magnifying the effect of the succeeding step.
For an overview of the coagulation cascade see the first
30 chapter of "Thombosis and Hemorrhage", J. Loscalzo and
A. Schafer, eds., Blackwell Scientific Publications,
Oxford, England (1994).

While efficient clotting limits the loss of blood
at an injury site, inappropriate formation of thrombi in
35 veins or arteries is a common cause of disability and
death. Abnormal clotting activity can result in and/or

from pathologies or treatments such as myocardial infarction, unstable angina, atrial fibrillation, stroke, renal damage, percutaneous transluminal coronary angioplasty, disseminated intravascular coagulation, sepsis, pulmonary embolism and deep vein thrombosis. The formation of clots on foreign surfaces of artificial organs, shunts and prostheses such as artificial heart valves is also problematic.

Stroke is a leading cause of death and a common cause of permanent disability. The acute focal cerebral ischemia resulting in the neurological deficits of stroke are most frequently caused by thromboembolism. Thrombi can be generated from cardiac sources and atheromas. *In situ* thrombosis can occur in the large, extracerebral brain-supplying vessels. Studies suggest a finite time interval after cerebral arterial occlusion beyond which significant irreversible neuronal damage and sustained neurological deficit occurs. See Chapter 14 of *Stroke Therapy: Basic, Preclinical, and Clinical Directions*, pp. 355-381, ed. L.P. Miller, Wiley-Liss, Inc. (1999).

Approved anticoagulant agents currently used in treatment of these pathologies and other thrombotic and embolic disorders include the sulfated heteropolysaccharides heparin and low molecular weight (LMW) heparin. These agents are administered parenterally and can cause rapid and complete inhibition of clotting by activation of the thrombin inhibitor, antithrombin III and inactivation of all of the clotting factors.

However, due to their potency, heparin and LMW heparin suffer drawbacks. Uncontrolled bleeding as a result of the simple stresses of motion and accompanying contacts with physical objects or at surgical sites is the major complication and is observed in 1 to 7% of patients receiving continuous infusion and in 8 to 14%

of patients given intermittent bolus doses. To minimize this risk, samples are continuously drawn to enable *ex vivo* clotting times to be continuously monitored, which contributes substantially to the cost of therapy and the patient's inconvenience.

Further, the therapeutic target range to achieve the desired level of efficacy without placing the patient at risk for bleeding is narrow. The therapeutic range is approximately 1 to less than 3 ug heparin/ml plasma which results in activated partial thromboplastin time (aPTT) assay times of about 35 to about 100 seconds. Increasing the heparin concentration to 3 ug/ml exceeds the target range and at concentrations greater than 4 ug/ml, clotting activity is not detectable. Thus, great care must be taken to keep the patient's plasma concentrations within the therapeutic range.

Another approved anticoagulant with slower and longer lasting effect is warfarin, a coumarin derivative. Warfarin acts by competing with Vitamin K dependent post-translational modification of prothrombin and other Vitamin K-dependent clotting factors.

The general pattern of anticoagulant action, in which blood is rendered non-clottable at concentrations only slightly higher than the therapeutic range is seen for warfarin as well as for heparin and LMW heparin.

In acute myocardial infarction (MI), the major objectives of thrombolytic therapy include early and sustained reperfusion of the infarcted vessel. Present therapy for acute MI includes both a plasminogen activator, such as tissue plasminogen activator (tPA) or streptokinase and an anticoagulant such as unfractionated heparin, low molecular weight heparin or direct thrombin inhibitors or antiplatelet agents such as aspirin or platelet glycoprotein IIb/IIIa blocker. See Topol, *Am Heart J*, 136, S66-S68 (1998). This

combination of therapies is based on the observation that clot formation and dissolution are dynamic processes and thrombin activity and generation continue after the formation of the occlusive thrombus and during and after dissolution of the clot. See Granger et al, *J Am Coll Cardiol*, 31, 497-505 (1998).

The optimal strategy for treatment of acute MI remains elusive and available agents and treatment protocols display both negative and positive characteristics. For example, fibrin-bound thrombin is insensitive to inhibition by heparin (Becker et al. in Chapter 6 of "Chemistry and Biology of Serpins", Plenum Press, New York (1997)) and thrombin activity exhibits a rebound increase following cessation of heparin therapy with an observed increase in reinfarction within 24 hours following discontinuation of heparin. See Watkins et al., *Catheterization and Cardiovascular Diagnosis*, 44, 257-264 (1998) and Granger, *Circulation*, 91, 1929-1935 (1995). Further, antiplatelet agents may be accompanied by bleeding or thrombocytopenia.

Also, numerous clinical trials have shown that high doses of thrombolytic agents lead to significant alteration in plasma hemostatic markers. See Rao et al., *J Clin Invest*, 101, 10-14 (1988); Bovill et al., *Ann Int Med*, 115, 256-265 (1991); Neuhaus et al., *J Am Coll Cardiol*, 19, 885-891 (1992). Although increasing concentrations of tPA lead to enhanced clot dissolution, the alteration in these hemostatic markers mirrors increased liabilities of thrombolytic therapy, particularly the incidence of severe bleeding.

In the case of thromboembolic stroke, thrombolytic therapy is employed early (within 3 hours) following the onset of stroke symptoms to prevent irreversible damage. Thrombolytic agents currently approved for reperfusion of ischemic and/or infarcted tissue include the plasminogen activators tPA, urokinase and streptokinase.

However, thrombolytic therapy is associated with a serious bleeding liability and a major concern of thrombolytic therapy in thromboembolic stroke is that the treatment will exacerbate the ischemic injury by
5 inducing hemorrhage.

Clearly, a need exists for antithrombotic agents efficacious in controlling thrombotic disorders while maintaining hemostatic functions.

SUMMARY OF THE INVENTION

10 An aspect of the invention is a method for treating an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment.

Another aspect of the invention is a method for
15 treating an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment in combination with a plasminogen activator.

Another aspect of the invention is a method of
20 reducing a required dose of a thrombolytic agent in treatment of an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment in combination with the thrombolytic agent.

25 Yet another aspect of this invention is a method for preventing thromboembolic stroke in an animal comprising administering an anti-Factor IX antibody or antibody fragment to an animal at risk for thromboembolic stroke.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of experimental results demonstrating the titration of normal human plasma with the murine anti-Factor IX mAbs BC1 and BC2.

5 Figure 2 is a graph of experimental results demonstrating the titration of normal human plasma with the murine anti-Factor IX mAbs 9E4(2)F4 and 11G4(1)B9.

10 Figure 3 is a graph of experimental results demonstrating the titration of normal human plasma with the murine anti-Factor X mAbs HFXHC and HFXLC and the murine anti-Factor XI mAb HFXI.

15 Figure 4 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on activated partial thromboplastin time (aPTT) at 60 minutes in a rat carotid thrombosis model.

20 Figure 5 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on prothrombin time at 60 minutes in a rat carotid thrombosis model.

Figure 6 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on occlusion of carotid artery flow in a rat carotid thrombosis model.

25 Figure 7 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on thrombus weight in a rat carotid thrombosis model.

30 Figure 8 is a histogram of experimental results demonstrating the effect of heparin, the murine Factor IX mab BC2, a chimeric Factor IX mab and humanized factor IX mAbs on aPTT at 60 minutes in a rat carotid thrombosis model.

35 Figure 9 is a histogram of experimental results demonstrating the effect of heparin, the murine Factor IX mab BC2, a chimeric Factor IX mab and humanized

factor IX mAbs on thrombus weight in a rat carotid thrombosis model.

Figure 10 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on tPA-mediated reperfusion.

Figure 11 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on carotid vessel patency.

Figure 12 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on time to restoration of blood flow.

Figure 13 demonstrates the effect of tPA on the hemostatic parameters, fibrinogen, plasminogen and antiplasmin.

Figure 14 demonstrates the effect of tPA, heparin and anti-Factor IX mab on aPTT.

Figure 15 demonstrates the effect of tPA, SB 249417, and the combination of tPA and SB 249417 on aggregate mean infarct volume in thromboembolic stroke.

DETAILED DESCRIPTION OF THE INVENTION

All publications, including but not limited to patents and patent applications, cited in the specification are herein incorporated by reference as though fully set forth.

The present invention provides a variety of antibodies, altered antibodies and fragments thereof directed against coagulation factors, which are characterized by self-limiting neutralizing activity. Preferably, the coagulation factor is from the intrinsic or common coagulation pathway. Most preferably, the anti-coagulation factor antibodies are anti-Factor IX, anti-Factor IXa, anti-Factor X, anti-Factor Xa, anti-Factor XI, anti-Factor XIa, anti-Factor VIII, anti-Factor VIIa, anti-Factor V, anti-Factor Va, anti-Factor VII, anti-Factor VIIa, anti-thrombin or anti-

prothrombin. Particularly preferred are anti-Factor IX antibodies. Exemplary anti-coagulation factor antibodies are the humanized monoclonal antibodies SB 249413, SB 249415; SB 249416, SB 249417, SB 257731 and 5 SB 257732 directed against human Factor IX, the chimeric monoclonal antibody ch α FIX directed against human Factor IX, the murine monoclonal antibodies BC1, BC2, 9E4(2)F4 and 11G4(1)B9 which are directed against human Factor IX and/or Factor IXa or the murine monoclonal antibodies 10 HFXLC and HFXI which are directed against human Factors X and XI, respectively. Particularly preferred is the anti-human Factor IX monoclonal antibody SB 249417.

The antibodies of the present invention can be prepared by conventional hybridoma techniques, phage 15 display combinatorial libraries, immunoglobulin chain shuffling and humanization techniques to generate novel self-limiting neutralizing antibodies. Also included are fully human mAbs having self-limiting neutralizing activity. These products are useful in therapeutic and 20 pharmaceutical compositions for thrombotic and embolic disorders associated with myocardial infarction, unstable angina, atrial fibrillation, stroke, renal damage, pulmonary embolism, deep vein thrombosis, percutaneous transluminal coronary angioplasty, 25 disseminated intravascular coagulation, sepsis, artificial organs, shunts or prostheses.

As used herein, the term "self-limiting neutralizing activity" refers to the activity of an antibody that binds to a human coagulation factor, 30 preferably from the intrinsic and common pathways, including Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa and V/Va, VII/VIIa and thrombin/prothrombin and inhibits thrombosis in a manner such that limited modulation of coagulation is produced. "Limited modulation of 35 coagulation" is defined as an increase in clotting time, as measured by prolongation of the activated partial

thromboplastin time (aPTT), where plasma remains clottable with aPTT reaching a maximal value despite increasing concentrations of monoclonal antibody. This limited modulation of coagulation is in contrast to plasma being rendered unclottable and exhibiting an infinite aPTT in the presence of increasing concentrations of heparin. Preferably, the maximal aPTT value of the methods of the invention are within the heparin therapeutic range. Most preferably, maximal aPTT is within the range of about 35 seconds to about 100 seconds which corresponds to about 1.5 times to about 3.5 times the normal control aPTT value. In one embodiment of the invention, aPTT is prolonged without significant prolongation of prothrombin time (PT).

The phrase "in combination with" refers to administration of one therapeutic agent before, after or concurrent with the administration of another therapeutic agent in a single course of treatment.

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, Fab' or F(ab')₂ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding an altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced
5 by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies
10 (immunoglobulins) can be determined by known methods in the art. For example Kabat *et al.* in "Sequences of Proteins of Immunological Interest", 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987) disclose rules for locating
15 CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame
20 or by means of an optional conventional linker sequence (*i.e.*, operatively linked). Preferably, it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (*i.e.*, homologous, where
25 the first and second altered antibodies are derived from the same source) or an additional (*i.e.*, heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not
30 limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)₂ (*i.e.*, a discrete part of an appropriate human constant region or framework
35 region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane

protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, Fab' or F(ab')₂ are used with their standard meanings. See, e.g., Harlow et al. in "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, (1988).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-

human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulins. In addition, framework support residues may be altered to preserve binding affinity. See, e.g., Queen *et al.*,
5 *Proc Natl Acad Sci USA*, 86, 10029-10032 (1989), Hodgson *et al.*, *Bio/Technology*, 9, 421 (1991).

The term "donor antibody" refers to a monoclonal or recombinant antibody which contributes the nucleic acid
10 sequences of its variable regions, CDRs or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and
15 neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a murine self-limiting neutralizing monoclonal antibody designated as BC2. Other suitable donor antibodies include the murine self-limiting
20 neutralizing monoclonal antibodies designated as BC1, 9E4(2)F4, 11G4(1)B9, HFXLC and HFXI.

The term "acceptor antibody" refers to monoclonal or recombinant antibodies heterologous to the donor antibody, which contributes all, or a portion, of the
25 nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably, a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity
30 determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat *et al.*, "Sequences of Proteins of Immunological Interest", 4th
35 Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three

heavy chain and three light chain CDRs or CDR regions in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs or both all heavy and all
5 light chain CDRs, if appropriate.

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences,
10 and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By "sharing the antigen binding specificity or
15 neutralizing ability" is meant, for example, that although mAb BC2 may be characterized by a certain level of self-limiting neutralizing activity, a CDR encoded by a nucleic acid sequence of BC2 in an appropriate structural environment may have a lower, or higher
20 activity. It is expected that CDRs of BC2 in such environments will nevertheless recognize the same epitope(s) as BC2.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the
25 amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by
30 at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen
35 specificity and high affinity, of the unmodified sequence. Exemplary analogs include silent mutations

which can be constructed, via substitutions, to create certain endonuclease restriction sites within or surrounding CDR-encoding regions.

5 Analogous may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide
10 desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

 The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other
15 plastic beads, polysaccharides, e.g., as used in the BIAcore (Pharmacia) system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy
20 metal atom or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

 For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species such as bovine, ovine, monkey, chicken, rodent
30 (e.g., murine and rat) may be employed to generate a desirable immunoglobulin upon presentment with a human coagulation factor, preferably factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or
35 thrombin/prothrombin or a peptide epitope therefrom. Conventional hybridoma techniques are employed to

provide a hybridoma cell line secreting a non-human mAb to the respective coagulation factor. Such hybridomas are then screened for binding using Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin bound to a streptavidin-coated plate. Alternatively, fully human mAbs can be generated by techniques known to those skilled in the art and used in this invention.

One exemplary, self-limiting neutralizing mAb of this invention is mAb BC2, a murine antibody which can be used for the development of a chimeric or humanized molecule. The BC2 mAb is characterized by a self-limiting inhibitory activity on clotting time. As measured by the aPTT assay, the effect of the BC2 mAb on clot time exhibits a maximal value of about 100 seconds. The BC2 mAb also binds Factor IXa, inhibits Factor IX to IXa conversion and inhibits Factor IXa activity. Divalent metal cofactors are required for activity, with the mAb exhibiting a greater preference for Ca^{2+} over Mn^{2+} . The observed IC_{50} in the aPTT assay is approximately 50 nM. The BC2 mAb exhibits a species cross-reactivity with rat and is of isotype IgG2a.

Other desirable donor antibodies are the murine mAbs, BC1, 9E4(2)F4 and 11G4(1)B9. These mAbs are characterized by a self-limiting inhibitory activity on clotting time. As measured by the aPTT assay, the effect of these mAbs on clot time exhibits a maximal value of about 90 to 100 seconds for 9E4(2)F4 and about 80 seconds for 11G4(1)B9. The BC1 mAb also binds Factor IXa, inhibits Factor IXa activity but does not inhibit Factor IX to IXa conversion. A metal cofactor is not required for its activity. The observed IC_{50} for BC1 in

the aPTT assay is approximately 35 nM. The BC1 mAb is of isotype IgG1.

Yet another desirable donor antibody characterized by a self-limiting inhibitory activity on clotting time is the murine mAb HFXLC. As measured by the aPTT assay, the effect of the HFXLC mAb on clot time exhibits a maximal value of about 50 to 60 seconds. The HFXLC mAb binds Factor X light chain, and inhibits Factor X/Xa activity. The observed IC_{50} in the aPTT assay is approximately 20 nM.

Yet another desirable donor antibody characterized by a self-limiting inhibitory activity on clotting time is the murine mAb, HFXI. As measured by the aPTT assay, the effect of the HFXI mAb on clot time exhibits a maximal value of about 100 seconds. The HFXLC mAb binds Factor XI and inhibits Factor XI/XIa activity. The observed IC_{50} in the aPTT assay is approximately 30 nM.

While not intending to be bound to any particular theory regarding the mechanism of action, these mAbs appear to regulate coagulation by a non-competitive or allosteric mechanism whereby only partial inhibition is achieved.

This invention is not limited to the use of the BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC, HFXI or their hypervariable (*i.e.*, CDR) sequences. Any other appropriate high-affinity antibodies characterized by a self-limiting neutralizing activity and corresponding CDRs may be substituted therefor. Identification of the donor antibody in the following description as BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC or HFXI is made for illustration and simplicity of description only.

The present invention also includes the use of Fab fragments or $F(ab')_2$ fragments derived from mAbs directed against the appropriate human coagulation factor or cofactor. These fragments are useful as agents having self-limiting neutralizing activity

against coagulation factors, preferably against Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain. An F(ab')₂ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. The mAbs BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC and HFXI and other similar high affinity antibodies, provide sources of Fab fragments and F(ab')₂ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and F(ab')₂ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')₂ fragments can be constructed via a combinatorial phage library (see, e.g., Winter *et al.*, *Ann Rev Immunol*, 12,433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks *et al.*, *Bio/Technology*, 10, 779-783 (1992), which are both hereby incorporated by reference in their entirety, wherein the Fd or v_H immunoglobulin from a selected antibody (e.g., BC2) is allowed to associate with a repertoire of light chain immunoglobulins, v_L (or v_K), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, v_H (or Fd), to form novel Fabs. Self-limiting neutralizing Factor IX Fabs can be obtained by allowing the Fd of mAb BC2 to associate with a repertoire of light chain immunoglobulins. Hence, one is able to recover neutralizing Fabs with unique sequences (nucleotide and amino acid) from the chain shuffling technique.

The mAb BC2 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions can be used to create restriction enzyme sites which facilitate insertion of mutagenized CDR and/or framework regions. These CDR-encoding regions can be used in the construction of the humanized antibodies of the invention.

The nucleic and amino acid sequences of the BC2 heavy chain variable region are listed in SEQ ID NOs: 5 and 7. The CDR sequences from this region are listed in SEQ ID NOs: 8, 9 and 10.

The nucleic and amino acid sequences of the BC2 light chain variable region are listed in SEQ ID NOs: 6 and 11. The CDR sequences from this region are listed in SEQ ID NOs: 12, 13 and 14.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated

nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, *e.g.*, chimeric or humanized antibodies or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions to the DNA sequences. See, T. Maniatis *et al.*, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory (1982), pp. 387-389. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for one hour. Alternatively, an exemplary stringent hybridization condition is 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, *i.e.*, about the size of a CDR.

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of a Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner such as a human framework or human immunoglobulin variable region.

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of coagulation factors may be designed to elicit enhanced binding with the same antibody.

The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde and the like. Such techniques are known in the art and are described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified by techniques known to those skilled in the art to enhance expression.

A preferred altered antibody contains a variable heavy and/or light chain peptide or protein sequence

having the antigen specificity of mAb BC2, e.g., the V_H and V_L chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the murine antibody molecule BC2 with the remaining sequences being derived from a human source, or a functional fragment or analog thereof.

In a further embodiment, the altered antibody of the invention may have attached to it an additional agent. For example, recombinant DNA technology may be used to produce an altered antibody of the invention in which the Fc fragment or CH₂ CH₃ domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having antigen specificity to a coagulation factor, preferably to Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin. The resulting protein may exhibit both antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain or a therapeutic characteristic if the fusion partner is itself a therapeutic protein or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains or any discrete fragment thereof, such as the Fab or F(ab')₂ fragments, a heavy chain dimer or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any

other molecule with the same specificity as the selected donor mAb, e.g., mAb BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC or HFXI. Such protein may be used in the form of an altered antibody or may be used in its unfused form.

5 Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise
10 immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or
15 thrombin/prothrombin antibodies described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made
20 in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the coagulation factor mAb (optionally modified as
25 described) or one or more of the heavy or light chain CDRs. The engineered antibodies of the invention exhibit self-limiting neutralizing activity.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected
30 human immunoglobulin or subtype or a chimeric antibody containing the human heavy and light chain constant regions fused to the coagulation factor antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a
35 conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology

to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Preferably, the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

A particularly preferred humanized antibody contains CDRs of BC2 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the Factor IX antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence, replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody.

Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable
5 acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g.,
10 treatment of thrombotic and embolic diseases in man.

Most preferably, the humanized antibodies have a heavy chain amino acid sequence as set forth in SEQ ID NO: 31, 52, or 89. Also most preferred are humanized antibodies having a light chain amino acid sequence as
15 set forth in SEQ ID NO: 44, 57, 62, 74, 78 or 99. Particularly preferred is the humanized antibody SB 249413 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 31 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 44.
20 Also particularly preferred is the humanized antibody SB 249415 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 57. Also particularly preferred is the humanized antibody SB
25 249416 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 62. Also particularly preferred is the humanized antibody SB 249417 where the heavy chain has the amino acid sequence
30 as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 74. Also particularly preferred is the humanized antibody SB 257731 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has
35 the amino acid sequence as set forth in SEQ ID NO: 78. Also particularly preferred is the humanized antibody SB

257732 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 89 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 99.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (*i.e.*, an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both. These substitutions could be supplied by the donor antibody or consensus sequences from a particular subgroup.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of this invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, *e.g.*, Angal *et al.*, *Mol Immunol*, 30, 105-108 (1993), Xu *et al.*, *J Biol Chem*, 269, 3469-3474 (1994), Winter *et al.*, EP 307434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies are useful in the prevention and treatment of thrombotic and embolic disorders, as discussed below.

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb BC2 or other suitable donor mAbs, *e.g.*, BC1, 9E4(2)F4, 11G4(1)B9, HFXLC, HFXI,

and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the murine antibody BC2, is conventionally cloned and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual", 2nd edition, Cold Spring Harbor Laboratory (1989). The variable heavy and light regions of BC2 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin, are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

Homologous framework regions of a heavy chain variable region from a human antibody are identified using computerized databases, e.g., KABAT®, and a human antibody having homology to BC2 is selected as the acceptor antibody. The sequences of synthetic heavy chain variable regions containing the BC2 CDR-encoding regions within the human antibody frameworks are designed with optional nucleotide replacements in the

framework regions to incorporate restriction sites. This designed sequence is then synthesized using long synthetic oligomers. Alternatively, the designed sequence can be synthesized by overlapping
5 oligonucleotides, amplified by polymerase chain reaction (PCR), and corrected for errors. A suitable light chain variable framework region can be designed in a similar manner.

A humanized antibody may be derived from the
10 chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be prepared
15 using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in
20 recombinant host cells, *e.g.*, COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this technique on other suitable Factor IX-specific or other coagulation factor-specific, self-limiting, neutralizing, high affinity, non-human antibodies.

25 A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or
30 secretion from, a host cell. Regulatory sequences include promoter sequences, *e.g.*, CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a
35 complementary antibody light or heavy chain. Preferably, this second expression vector is identical

to the first except with respect to the coding sequences and selectable markers, in order to ensure, as much as possible, that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by an appropriate assay such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the pUC series of cloning vectors, such as pUC19, which is commercially available from supply houses, such as Amersham or Pharmacia, may be used. Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance) and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected

regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above-described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g., replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in

the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3) and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, *supra*.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., *Immunol Rev*, 130, 151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g.

Miller *et al.*, *Genetic Engineering*, 8, 277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods
5 required to produce the host cells of the invention, and culture methods necessary to produce the altered
antibody of the invention from such host cells are all conventional techniques. Likewise, once produced, the
altered antibodies of the invention may be purified from
10 the cell culture contents according to standard procedures of the art, including ammonium sulfate
precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are
within the skill of the art and do not limit this
15 invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic
animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's
20 casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired
recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro*
25 activity by use of an appropriate assay. Presently, conventional ELISA assay formats are employed to assess
qualitative and quantitative binding of the engineered antibody to Factor IX or to other appropriate
coagulation factors. Additionally, other *in vitro*
30 assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to
evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the procedures described for humanized
35 antibodies prepared from BC2, one of skill in the art may also construct humanized antibodies from other donor

antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody.

5 Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies may effectively treat a human for coagulation factor-
10 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

This invention also relates to a method for inhibiting thrombosis in an animal, particularly a human, which comprises administering an effective dose
15 of an anti-coagulation factor monoclonal antibody having self-limiting neutralizing activity in combination with a plasminogen activator. Combination therapy enhances thrombolysis at sub-optimal concentrations of plasminogen activator decreasing the time to restoration
20 of blood flow and increasing the frequency and total duration of vessel reperfusion. In contrast to heparin, combination therapy does not significantly perturb normal hemostatic functions and spares fibrinogen, plasminogen and alpha-2-antiplasmin levels.

25 Accordingly, this invention also relates to a method of reducing a required dose of a thrombolytic agent in treatment of thrombosis in an animal comprising administering an anticoagulant specifically targeting a component of the intrinsic coagulation pathway in
30 combination with the thrombolytic agent.

Preferably, the coagulation factor is from the intrinsic or common coagulation pathway. Most preferably, the anti-coagulation factor monoclonal antibody is an anti-Factor IX, anti-Factor IXa, anti-
35 Factor X, anti-Factor Xa, anti-Factor XI, anti-Factor XIa, anti-Factor VIII, anti-Factor VIIIa, anti-Factor V,

anti-Factor Va, anti-Factor VII, anti-Factor VIIa, anti-thrombin or anti-prothrombin. The mAb can include one or more of the engineered antibodies or altered antibodies described herein or fragments thereof.

5 Preferably, the plasminogen activator is tPA, streptokinase, urokinase. Particularly preferred is tPA. Also preferred are tPA variants as described in, e.g., Tachias and Madison, *J Biol Chem*, 272, 14580-14585 (1997); Fujise *et al.*, *Circulation*, 95, 715-722 (1997);
10 Coombs *et al.*, *J Biol Chem*, 273, 4323-4328 (1998); Van de Werf, *et al.*, *Am Heart J*, 137, 786-791 (1999) and streptokinase and urokinase variants, such as single-chain urokinase plasminogen activator, acylated plasminogen streptokinase activator complex,
15 staphylokinase and plasminogen activators of vampire bat origin.

Alternatively, acetylsalicylic acid can be administered in combination with the anti-coagulation factor monoclonal antibody. In some cases, combination
20 therapy lowers the therapeutically effective dose of anti-coagulation factor monoclonal antibody.

The therapeutic response induced by the use of the molecules of this invention is produced by the binding to the respective coagulation factor and the
25 subsequent self-limiting inhibition of the coagulation cascade. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for persons susceptible to or experiencing abnormal clotting
30 activity associated with, but not limited to, myocardial infarction, unstable angina, atrial fibrillation, stroke, renal damage, pulmonary embolism, deep vein thrombosis and artificial organ and prosthetic implants. A particularly preferred use is in myocardial
35 infarction.

Another preferred use is in treatment of post-thromboembolic induced ischemia. Accordingly, this invention also relates to a method for treating an animal post-thromboembolic induced ischemia comprising
5 administering an anti-Factor IX antibody or antibody fragment. The antibody or a fragment can be administered post-embolus, *i.e.*, after a clot originating anywhere in the vasculature has traveled into the cerebral vasculature and lodges, blocking
10 and/or reducing blood flow and causing ischemia. The antibody or a fragment can also be administered post-stroke, *i.e.*, after recognition on the part of an individual or an observer of impaired neurological function resulting from embolization. Further, this
15 invention relates to a method for treating an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment in combination with a plasminogen activator. The antibody or a fragment and plasminogen activator can
20 be administered post-embolus or post-stroke. These treatment methods result in maintenance of vascular perfusion in collateral vessels. The post-injury treatment methods of the invention mitigate the consequences of prolonged ischemia such as continuing
25 pathological thrombosis in the ischemic bed, which can result in the growth of infarcted tissue and greater neurological deficits.

Further, this invention relates to a method for reducing a required dose of a thrombolytic agent in
30 treatment of an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody in combination with the thrombolytic agent.

Another preferred use is prophylactic administration to an animal susceptible to
35 thromboembolic stroke. Thus, the invention also relates to a method for preventing thromboembolic stroke in an

animal comprising administering an anti-Factor IX antibody to an animal at risk for thromboembolic stroke. Those at risk for thromboembolic stroke include, but are not limited to, patients susceptible to atrial
5 fibrillation or those undergoing surgical interventions or other pro-coagulant invasive techniques.

The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human
10 mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for treatment of abnormal
15 clotting conditions from about 1 day to about 3 weeks, or as needed. This represents a considerable advance over the currently used anticoagulants heparin and warfarin. The dose and duration of treatment relates to the relative duration of the molecules of the present
20 invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agents of the invention may be any suitable route which
25 delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, plasminogen activator and pharmaceutical compositions of the invention are particularly useful for parenteral administration, *i.e.*, subcutaneously,
30 intramuscularly, intravenously or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (*e.g.*, humanized) antibody of the invention and plasminogen activator as active
35 ingredients in a pharmaceutically acceptable carrier. Alternatively, the pharmaceutical compositions of the

invention could also contain acetylsalicylic acid. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 mg to about 30 mg and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known

or will be apparent to those skilled in the art and are described in more detail in, for example, "Remington's Pharmaceutical Science", 15th ed., Mack Publishing Company, Easton, Pennsylvania.

5 It is preferred that the therapeutic agents of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat a
10 thrombotic or embolic disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.* or *i.m.* Such dose may, if
15 necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the thrombotic response.

 The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage
20 and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

25

 The present invention will now be described with reference to the following specific, non-limiting examples.

Example 1Preparation and Screening of Anti-Factor IX Monoclonal
Antibodies

Female Balb/C mice were injected with human factor
5 IX purified as described in Jenny, R. *et al.*, *Prep
Biochem*, 16, 227-245 (1986). Typically, each mouse
received an initial injection of 100 ug protein
dissolved in 0.15 mL phosphate-buffered saline (PBS) and
mixed with 0.15 mL complete Freund's adjuvant. Booster
10 immunizations of 50 ug protein in 0.15 mL PBS with 0.15
mL incomplete Freund's adjuvant were given approximately
biweekly over a 2-3 month period. After the final
boost, the mouse received 50 ug of Factor IX in PBS
three days before spleen/myeloma cell fusions. Spleen
15 cells were isolated from an immunized mouse and fused
with NS-1 myeloma cells (Kohler, G. *et al.*, *Eur J
Immunol*, 6, 292-295 (1976)) using polyethylene glycol as
described by Oi, V.T. *et al.* in "Selected Methods in
Cellular Immunology," Mishell, B.B. and Shigii, S.M.,
20 eds., Freeman Press, San Francisco. Following the
fusion, the cells were resuspended in RPMI 1640 media
containing 10% fetal calf sera and aliquots were placed
in each well of four 24-well plates containing 0.5 mL of
peritoneal lavage cell-conditioned media. On the
25 following day, each well received 1.0 mL of 2×10^{-4} M
hypoxanthine, 8×10^{-7} M aminopterin and 3.2×10^{-5} M
thymidine in RPMI 1640 media containing 10% fetal calf
sera. The cells were fed every 3-4 days by removing
half of the media and replacing it with fresh media
30 containing 1×10^{-4} M hypoxanthine and 1.6×10^{-5} M
thymidine.

Approximately two weeks later, 1.0 mL of hybridoma
medium was removed from each well and tested for anti-
Factor IX antibodies using an ELISA assay as described
35 by Jenny, R.J. *et al.* in *Meth Enzymol*, 222, 400-416
(1993). Briefly, factor IX was immobilized onto

plastic wells of 96-well microtiter plates. Hybridoma supernatants or dilutions of purified antibody were then incubated in the wells. The wells were washed and the presence of antibody-antigen complexes detected with a
5 goat anti-murine immunoglobulin second antibody conjugated to horseradish peroxidase and the chromogenic substrate *o*-dianisidine.

Wells containing anti-Factor IX antibodies were subcloned by limiting dilution and grown in 96-well
10 plates. Supernatant from the cloned hybridoma cell cultures were screened for antibody to Factor IX by the ELISA assay described above and cells from positive hybridomas were expanded, frozen, stored in liquid nitrogen and then grown as ascitic tumors in mice.

15

Example 2

Self-Limiting Effect of Anti-Coagulation Factor Antibodies in Coagulation

The effect of increasing concentrations of anti-coagulation factor antibodies on activated partial
20 thromboplastin time (aPTT) of human plasma was determined in a fibrometer (Becton-Dickinson Microbiology Systems, Cockeysville, Maryland) using Baxter reference procedure LIB0293-J, 3/93 revision
25 (Baxter Scientific, Edison, New Jersey).

Prior to the start of the experiment, 2 to 3 mL of 0.02 M CaCl₂ in a 5 mL tube were placed into the heating chamber of the fibrometer. Human plasma samples were either freshly drawn and kept on ice or reconstituted
30 per the manufacturer's recommendation from Hemostasis Reference Plasma (American Diagnostics, Greenwich, Connecticut).

Unfractionated heparin from porcine intestinal mucosa (Sigma Chemical, St. Louis, Missouri), low
35 molecular weight heparin from porcine intestinal mucosa (Lovenox®, enoxaparin sodium, Rhone-Poulenc Rorer

Pharmaceuticals, Colleagueville, Pennsylvania) or mAb anticoagulants were prepared as approximately 50 uM stock solutions and serially diluted directly into the test plasma. A blank containing plasma without
5 anticoagulant was included as a reference.

Two fibroTube® fibrometer cups were filled with 100 ul test plasma or 100 ul test plasma with anticoagulant and 125 ul of actin activated cephaloplastin reagent (Actin reagent, from rabbit brain cephalin in ellagic
10 acid, available from Baxter Scientific), respectively and placed in the fibrometer wells at 37°C.

After one minute, 100 ul of actin reagent was transferred to a plasma-containing cup and the contents mixed several times with a pipette. After a 3 minute
15 incubation, 100 ul of CaCl₂, prewarmed at 37°C, was added to the plasma-actin reagent mixture using a Automatic Pipette/Timer-trigger (Becton-Dickinson). The clotting times were noted and the results in Fig. 1 are presented as clotting times as a function of final concentrations
20 of anticoagulant in the total assay volume of 300 ul. The nominal concentration of Factor IX in the assay is 30-40 nM.

The results shown in Fig. 1 demonstrate the effect of increasing concentrations of the murine anti-Factor
25 IX mAbs BC1 and BC2 on aPTT clotting times. Both mAbs inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. The IC₅₀ values are similar at ~35 nM and ~50 nM for BC1 and BC2, respectively, but the difference in the maximum response
30 to the two antibodies is marked. Saturating concentrations of BC1 increases the aPTT by about 50% to ~40 sec. BC2, on the other hand, increases the aPTT by 3.5-fold to about 90 sec. The therapeutic target zone used in anticoagulant therapy with heparin is
35 highlighted. The results indicate that the two mAbs bracket the heparin therapeutic aPTT range.

The properties of mAbs BC1 and BC2 are summarized in Table I. Each of the BC mAbs recognizes both the zymogen, Factor IX, as well as the active protease, Factor IXa, but only BC2 is capable of blocking both zymogen activation as well as protease activity. BC1 and BC2 were found to cross-react with Cynomologous monkey Factor IX. Additionally, BC2 also cross-reacted with rat Factor IX.

10 Table I. Summary of *in vitro* Properties of Anti-Factor IX mAbs

	BC1	BC2
Binds Factor IX	yes	yes
Binds Factor IXa	yes	yes
Inhibits IX to IXa conversion	no	yes
Inhibits IXa activity in Xase complex	yes	yes
Cofactor requirement	none	divalent metals Ca ²⁺ > Mn ²⁺
$\frac{\text{aPTTmax}}{\text{aPTTnormal}} \times 100\%$	150	350
IC ₅₀ , nM	~35	~50
Species cross-reactivity	monkey	rat, monkey
Isotype	IgG1	IgG2a

The results shown in Fig. 2 demonstrate the effect of increasing concentrations of the anti-Factor IX mAbs 9E4(2)F4 and 11G4(1)B9 on aPTT clotting times. The plasma for the assay was diluted to one-half the normal concentration, giving an initial aPTT of 45 seconds. Both mAbs inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. Saturating concentrations of 9E4(2)F4 and 11G4(1)B9 increases the aPTT to ~90 to 100 seconds for 9E4(2)F4 and to ~80 seconds for 11G4(1)B9. The results indicate that the two mAbs are at the upper end of the heparin therapeutic aPTT range.

The results shown in Fig. 3 demonstrate the effect of increasing concentrations of the anti-Factor X mAbs HFXLC (vs. light chain epitope), HFXHC (vs. heavy chain epitope) and the anti-Factor XI mAb HFXI on aPTT clotting times. These mAbs were obtained from Enzyme Research Laboratories (South Bend, IN). The mAbs HFXLC and HFXI inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. The IC_{50} value for HFXLC is ~40 nM; saturating concentrations increase the aPTT to ~60 seconds. The IC_{50} value for HFXI is ~20 nM; saturating concentrations increase the aPTT to ~100 seconds. The results indicate that HFXLC is within the heparin therapeutic aPTT range while HFXI falls at the upper end of the heparin therapeutic range. The mAb HFXHC had no effect on aPTT clotting times.

Self-limiting prolongation of the aPTT was also observed with antibodies to Factor VIII, the cofactor to Factor IXa. For example, the anti-human Factor VIII antibody, SAF8C-IG, purchased from Affinity Biologicals, Inc., increased the aPTT to a maximum of about 65 sec. Half-maximal prolongation of the aPTT was achieved with about 100 nM antibody.

Example 3**Efficacy of murine Factor IX mAbs in Rat Thrombus Model**

In order to evaluate the efficacy of anti-Factor IX antibodies in prevention of arterial thrombosis, the rat carotid artery thrombosis model as reported by Schumacher *et al.* in *J Cardio Pharm*, 22, 526-533 (1993) was adapted. This model consists of segmental injury to the carotid endothelium by oxygen radicals generated by FeCl₃ solution applied on the surface of the carotid artery.

In brief, rats were anesthetized with pentobarbitone sodium, the jugular vein cannulated for intravenous injections and the left femoral artery cannulated for blood pressure and heart rate monitoring. The carotid artery was isolated by aseptic technique via a surgical incision in the neck and equipped with a magnetic flow probe for blood flow measurement. After a period of stabilization, baseline parameters were established for the following variables: carotid blood flow, arterial pressure, heart rate, activated partial thromboplastin time (aPTT) and prothrombin time (PT). Thereafter, a premeasured Whatman filter paper soaked in 50% FeCl₃ solution was placed on the carotid artery for 15 minutes for complete injury of the underlying endothelial cells. After removal of the FeCl₃ soaked paper, the experiment was followed to completion over 60 minutes. At the end of the experiment, the carotid thrombus was extracted from the carotid artery and weighed.

All agents were administered 15 minutes prior to the onset of carotid injury. The following treatments were examined and compared to the Factor IX mAb BC2.

1. Heparin: 15, 30, 60 or 120 U/kg bolus, followed by infusion of 0.5, 1, 2 or 4 U/kg/min, respectively over 60 minutes

2. Acetylsalicylic acid (ASA, aspirin): 5 mg/kg bolus
3. Anti-Factor IX mAb BC2: 1, 3 or 6 mg/kg bolus, followed by infusion 0.3, 1, or 2 ug/kg/min, respectively over 60 minutes
4. Heparin: 30U/kg bolus + 1U/kg/min + ASA at 5 mg/kg
5. Anti-Factor IX mAb BC2: 1 mg/kg + 0.3 ug/kg/min + ASA at 5 mg/kg

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Figs. 4 and 5 demonstrate the comparative pharmacology of the anti-coagulant/thrombotic regimens by showing the effect of heparin, ASA and Factor IX mAb BC2 on aPTT (Fig. 4) and PT (Fig. 5).

15

The key index for bleeding diathesis, aPTT, was used as the primary criterion for evaluation of efficacy versus bleeding liabilities of the anti-coagulant/thrombotic agents used in the study. The results in Fig. 4 demonstrate the dose-dependent prolongation of aPTT by heparin with maximal prolongation of the clotting time, beyond the test limit, at the two higher doses. ASA alone did not significantly increase aPTT but in combination with heparin, a marked synergistic effect was observed. The Factor IX mAbs had a modest effect on aPTT and even at the highest dose, the increase in clotting time did not exceed the 3-fold limit of standard anti-coagulant practiced clinically. Most notably, the low dose of Factor IX mAb BC2 in combination with ASA did not change the aPTT.

20
25
30

In Fig. 5, the data indicate that PT was also significantly prolonged by heparin, at the two higher doses, and by the ASA + heparin combination, but not by any of the Factor IX mAb doses alone or in combination with ASA.

35

The effect of heparin, ASA and Factor IX mAb on carotid artery occlusion is shown in Fig. 6. The results indicate that the carotid arteries of all of the vehicle-treated animals occlude in response to the injury. Heparin dose dependently inhibited the occlusion of the carotid artery. At the highest dose, heparin completely prevented the occlusion of the carotid artery; at this dose however, no coagulation could be initiated. ASA alone had only a minor effect on carotid occlusion. ASA in combination with heparin also failed to completely prevent carotid occlusion. Factor IX mAb completely blocked carotid occlusion at the two higher doses, which have not prolonged coagulation beyond the clinically desired target. The lower dose of Factor IX mAb, that largely failed to secure patency alone, demonstrated complete inhibition of carotid occlusion when administered in combination with ASA.

The effect of heparin, ASA and Factor IX mAb on thrombus weight is shown in Fig. 7. Heparin dose-dependently reduced thrombus mass in the carotid artery. However, some residual thrombus was still found in the carotid artery in spite of complete blockade of coagulation. ASA alone or in combination with heparin (30 U/kg regimen) had only a partial effect on thrombus weight. Factor IX mAb dose-dependently reduced thrombus mass and the high dose virtually prevented completely thrombus formation. Moreover, the combination of the low dose anti-Factor IX mAb and ASA, a regimen that completely prevented carotid occlusion without adversely affecting the coagulation indices, completely prevented thrombus formation.

The studies conducted in the rat carotid thrombosis model clearly demonstrate the efficacy of Factor IX mAb in prevention of thrombosis in a highly thrombogenic arterial injury model. Most notably, the efficacy of

the Factor IX mAb was demonstrated within the desired therapeutic anticoagulant target defined by the aPTT. Furthermore, heparin, the current standard anticoagulant, reached efficacy comparable to Factor IX mAb only at doses that severely compromised coagulation to the extent of producing non-coagulable blood. Interestingly, the observed potentiation and synergy acquired by ASA joint treatment with heparin was also demonstrated when ASA was given with anti-Factor IX mAb. However, unlike the combination of heparin and ASA which resulted in potentiation of both the anti-thrombotic and anti-coagulant effects, the combination of Factor IX mAb and ASA resulted in potentiation of the anti-thrombotic efficacy with no consistent effect on *ex vivo* blood coagulation parameters. Taken together, the data show a superior antithrombotic capacity of Factor IX mAb compared to heparin, ASA or a combination of heparin and ASA.

20

Example 4

Scanning Electron Microscopy of Rat Thrombosis Model

Segments of rat carotid artery were collected from sham, ferric chloride only and ferric chloride + 6 mg/kg Factor IX antibody, 3/group, 15 minutes after application of ferric chloride. The arteries were fixed by perfusion with formaldehyde and ligated above and below the lesioned area. Fixed arteries were dehydrated, incubated in hexamethyldisilazane and dried in a desiccator. Dried arteries were opened lengthwise, placed on Scanning Electron Microscopy (SEM) stubs and sputter coated with gold.

SEM of sham arteries revealed an essentially normal endothelium with rare scattered platelets. There were a few breaks in the endothelium, probably as a result of mechanical damage during surgery and the underlying basement membrane was covered by a carpet of platelets.

No evidence of thrombus formation was observed in the sham rats.

SEM of the arteries treated with ferric chloride revealed large mural thrombi which occupied a large portion of the lumen of the vessel. The thrombi were composed of aggregated platelets, red blood cells and amorphous and fibrillar proteinaceous material. The proteinaceous material is consistent with fibrin. The endothelium of the arteries was mostly obscured by the large thrombi. Where visible, the endothelium overlying the region treated with ferric chloride was covered by numerous adherent platelets and amorphous proteinaceous material.

SEM of the arteries treated with ferric chloride from rats also treated with Factor IX antibody, revealed the lumen of the vessels to be largely free of thrombus. The endothelium overlying the region treated with ferric chloride showed extensive damage and some areas were covered by adherent platelets and platelet aggregates but there was little or no proteinaceous material.

Example 5

Anti-Factor IX mAb BC2 Heavy and Light Chain cDNA

Sequence Analysis

Total RNA was purified by using TriReagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. RNA was precipitated with isopropanol and dissolved in 0.5% SDS and adjusted to 0.5M NaCl. Poly A⁺ RNA was isolated with Dynabeads Oligo (dT)₂₅ (Dynal A.S., Lake Success, NY) according to the manufacturer's protocol. Poly A⁺ RNA was eluted from the beads and resuspended in TE buffer. Twelve aliquots of 100 ng of RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming. For the heavy chain, PCR

amplifications of 6 RNA/DNA hybrids were carried out for 25 cycles using a murine IgG2a hinge primer (SEQ ID NO: 1) and a heavy chain signal sequence primer (SEQ ID NO: 2). Similarly, for the light chain, PCR amplifications of 6 RNA/DNA hybrids were carried out for 25 cycles using a murine kappa primer (SEQ ID NO: 3) and a degenerate light chain signal sequence primer (SEQ ID NO: 4). The PCR products from each of the 12 amplifications were ligated in a PCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01). Colonies of recombinant clones were randomly picked and minipreparations of plasmid DNA were prepared using an alkaline extraction procedure described by Birnboim and Doly in *Nucl. Acids Res.* 7, 1513 (1979). The isolated plasmid DNA was digested with *EcoRI* and analyzed on a 0.8% agarose gel. Double-stranded cDNA inserts of the appropriate size, *i.e.*, ~700 bp for the heavy chain and ~700 bp for the light chain, were sequenced by a modification of the Sanger method. The sequence of all 12 of the heavy and light chains were compared to generate a consensus BC2 heavy chain variable region sequence (SEQ ID NO: 5) and consensus BC2 light chain variable region sequence (SEQ ID NO: 6).

Sequence analysis of the BC2 heavy chain variable region cDNA revealed a 363 nucleotide open reading frame encoding a 121 amino acid sequence (SEQ ID NO: 7). The heavy chain CDR1, 2 and 3 sequences are listed in SEQ ID NOs: 8, 9 and 10, respectively.

Sequence analysis of the BC2 light chain variable region cDNA revealed a 321 nucleotide open reading frame encoding a 107 amino acid sequence (SEQ ID NO: 11). The light chain CDR1, 2 and 3 sequences are listed in SEQ ID NOs: 12, 13 and 14, respectively.

Example 6**Humanized Antibodies**

Six humanized antibodies designated SB 249413, SB 249415, SB 249416, SB249417, SB 257731 and SB 257732 were designed to contain the murine CDRs described above in a human antibody framework.

SB 249413

SB 249413 contains the heavy chain F9HZHC 1-0 and the light chain F9HZLC 1-0. The synthetic variable region humanized heavy chain F9HZHC 1-0 was designed using the first three framework regions of the heavy chain obtained from immunoglobulin RF-TS3'CL (Capra, J.D. *et al.*, *J. Clin. Invest.* 86, 1320-1328 (1990) identified in the Kabat database as Kabpro:Hhc10w) and the BC2 heavy chain CDRs described previously. No framework amino acids substitutions which might influence CDR presentation were made. Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 15, 16, 17 and 18) which, when annealed and extended, code for the amino acids representing the heavy chain variable region through and including CDR3 (SEQ ID NOs: 19 and 20). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 21 and 22) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *SpeI*, *KpnI* restriction digest. A second DNA fragment coding for the campath signal sequence including the first five amino acids of the variable region (SEQ ID NOs: 23 and 24) was made by PCR amplification of the appropriate region of a construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with two primers (SEQ ID NOs: 26 and 27) and digesting with the restriction enzymes *EcoRI* and *SpeI*. The two fragments generated were ligated into an *EcoRI*, *KpnI* digested pFHZHC2-6pCD mammalian cell expression vector which contained the remainder of a human consensus

framework 4 and IgG1 constant region. The vector contained a single amino acid mutation of the pFHZHC2-3pCD vector described in published International Patent Application No. WO94/05690. The final residue of framework 2 (residue 49) was mutated from Ser to Ala by digesting pFHZHC2-3pCD with *Xba*I and *Eco*R5 and inserting a linker generated from two synthetic oligonucleotides (SEQ ID NOs: 28 and 29). The sequence of the F9HZHC 1-0 insert is shown in SEQ ID NOs: 30 and 31.

The synthetic variable region humanized light chain F9HZLC 1-0 was designed using the framework regions of the human light chain obtained from immunoglobulin LS8'CL (Carmack *et al.*, *J. Exp. Med.* 169, 1631-1643 (1989) identified in the Kabat database as Kabpro:Hkl318) and the BC2 light chain CDRs described previously. No framework amino acids substitutions which might influence CDR presentation were made. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 32 and 33) which, when annealed and extended, code for amino acids representing the light chain variable region (SEQ ID NOs: 34 and 35). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 36 and 37) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01), and isolated from a *Sca*I, *Sac*II restriction digest. A second DNA fragment coding for the campath signal sequence including the first two amino acids of the variable region (SEQ ID NOs: 38 and 39) was made by PCR amplification of the the appropriate region of a construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with the two primers (SEQ ID NOs: 26 and 40) and digesting with the restriction enzymes *Eco*RI and *Sca*I. The two fragments generated were ligated into an *Eco*R1, *Sac*II digested pFHZLC1-2pCN mammalian cell expression vector which contained the remainder of a human framework 4 and kappa

constant region. The vector contained a single amino acid mutation of the pFHZLC1-1pCN vector described in published International Patent Application No. WO94/05690. A framework 2 residue was mutated from Ser to Pro by digesting pFHZLC1-pCN with *Sma*I and *Kpn*I and inserting a linker generated from two synthetic oligonucleotides (SEQ ID NOs: 41 and 42). The sequence of the F9HZLC 1-0 insert is shown in SEQ ID NOs: 43 and 44.

10 **SB 249415**

SB 249415 contains the heavy chain F9HZHC 1-1 and the light chain F9HZLC 1-1. These heavy and light chain constructs are based on F9HZHC 1-0 and F9HZLC 1-0, respectively, however, they have framework amino acid substitutions which can influence CDR presentation.

F9HZHC 1-1 has three framework amino acid substitutions which might influence CDR presentation. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 45 and 46), which when annealed and extended, code for amino acids representing the altered portion of the heavy chain variable region altered (SEQ ID NOs: 47 and 48). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 49 and 50), ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *Eco*NI, *Kpn*I restriction digest. This fragment was ligated into *Eco*NI, *Kpn*I digested F9HZHC1-0 (SEQ ID NO: 30) vector. The sequence of the F9HZHC 1-1 insert is shown in SEQ ID NOs: 51 and 52.

F9HZLC 1-1 has four framework amino acids substitutions which can influence CDR presentation. Two synthetic oligonucleotides were generated (SEQ ID NOs: 53 and 54), which when annealed, have *Kpn*I and *Bam*HI cohesive ends, and code for amino acids representing the altered portion of the light chain variable region (SEQ ID NO: 55). F9HZLC 1-0 (SEQ ID NO: 43) was digested

with the restriction enzymes *KpnI* and *BamHI* and ligated to the synthetic DNA. The sequence of the F9HZLC 1-1 insert is shown in SEQ ID NOs: 56 and 57.

SB 249416

5 SB 249416 contains the heavy chain F9HZHC 1-1 (described above) (SEQ ID NO: 52) and the light chain F9HZLC 1-2. The light chain construct is based on F9HZLC 1-1, however, it has one additional framework amino acid substitution which can influence CDR
10 presentation.

Two synthetic oligonucleotides were generated (SEQ ID NOs: 58 and 59), which when annealed, have *BamHI* and *XbaI* cohesive ends and code for amino acids representing the altered portion of the light chain variable region
15 (SEQ ID NO: 60). F9HZLC 1-1 (SEQ ID NO: 56) vector was digested with the restriction enzymes *BamHI* and *XbaI* and ligated to the synthetic DNA. The sequence of the F9HZLC 1-2 insert is shown in SEQ ID NOs: 61 and 62.

SB 249417

20 SB 249417 contains the heavy chain F9HZHC 1-1 (described above) (SEQ ID NO: 52) and the light chain F9HZLC 2-0. A F9HZLC 2-0 synthetic variable region humanized light chain was designed using the framework regions of the human light chain obtained from
25 immunoglobulin REI (Palm and Hilschmann, *Z. Physiol. Chem.* 354, 1651-1654 (1973) identified in the Kabat database as Kabpro: HKL111) and the BC2 light chain CDRs described previously. Five amino acid consensus human substitutions were introduced. Six framework amino
30 acids murine substitutions which can influence CDR presentation were made. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 63 and 64) which, when annealed and extended, code for amino acids representing the light chain variable region (SEQ ID
35 NOs: 65 and 66). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 67 and 68), ligated into

the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *ScaI*, *SacII* restriction digest. A second DNA fragment coding for the campath signal sequence including the first two amino acids of the variable region (SEQ ID NO: 38) was made by PCR amplification of the the appropriate region of a construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with two primers (SEQ ID NOs: 26 and 69) and digesting with the restriction enzymes *EcoRI* and *ScaI*. A third DNA fragment encoding the remainder of a human framework 4 (SEQ ID NO: 70) and having *SacII* and *NarI* cohesive ends was generated by annealing two synthetic oligonucleotides (SEQ ID NOs: 71 and 72). F9HZLC 1-0 (SEQ ID NO: 43) was digested with the restriction enzymes *EcoRI* and *NarI* and ligated to the three DNA fragments. The sequence of the F9HZLC 2-0 insert is shown in SEQ ID NOs: 73 and 74.

SB 257731

SB 257731 contains the heavy chain F9HZHC 1-1 (SEQ ID NO: 52) and the light chain F9HZLC 1-3, a single amino acid mutation of F9HZLC 1-2 (SEQ ID NO: 62). F9HZLC 1-2 was PCR amplified with two primers (SEQ ID NOs: 26 and 69) and digested with the restriction enzymes *EcoRI* and *ScaI*. A 94 bp fragment (SEQ ID NOs: 75 and 76) was isolated. The fragment was ligated into *EcoRI*, *ScaI* digested F9HZLC 1-2 vector to produce the light chain construct F9HZLC 1-3. The sequence of the F9HZLC 1-3 insert is shown in SEQ ID NOs: 77 and 78.

SB 257732

SB 257732 contains the synthetic variable region humanized heavy chain F9HZHC 3-0 and light chain F9HZLC 3-0. Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 79, 80, 81 and 82) which, when annealed and extended, code for the amino acids representing the heavy chain variable region being

altered (SEQ ID NOs: 83 and 84). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 85 and 86), ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *StuI*, *KpnI* restriction digest. The isolated fragment was ligated into *StuI*, *KpnI* digested F9HZHC1-1 (SEQ ID NO: 52) vector. This vector was then digested with *EcoRI*, *SpeI* to remove the signal sequence. A DNA fragment coding for the campath signal sequence (SEQ ID NO: 23) including the first five amino acids of the variable region was made by PCR amplification of F9HZHC1-0 with two primers (SEQ ID NOs: 26 and 87) and digesting with the restriction enzymes *EcoRI* and *SpeI*. The fragment generated was ligated into the vector. The sequence of the F9HZHC3-0 insert is shown in SEQ ID NOs: 88 and 89.

Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 90, 91, 92 and 93) which, when annealed and extended, code for amino acids representing the light chain variable region (SEQ ID NOs: 94 and 95). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 96 and 97) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01), and isolated from a *ScaI*, *NarI* restriction digest. The isolated fragment was ligated into *ScaI*, *NarI* digested F9HZLC1-3 (SEQ ID NO: 77) vector. The sequence of the F9HZLC3-0 insert is shown in SEQ ID NOs: 98 and 99.

The humanized anti-Factor IX mAbs were expressed in CHO cells. A DG-44 cell line adapted for suspension growth in serum-free medium was grown in 100ml of protein-free medium containing 1X nucleosides and 0.05% F68 in 250 ml disposable sterile erlenmeyer flasks (Corning) on a Innova 2100 platform shaker (New Brunswick Scientific) at 150 rpm at 37°C in a 5% CO₂, 95% air humidified incubator. These cells were passaged at 4 X 10⁵ cells/ml twice weekly. 15 ug each of the pCN-Lc-Light Chain and pCD-Hc-heavy chain vectors were

linearized by digestion with *Not1*, co-precipitated under sterile conditions and resuspended in 50ul of 1X TE buffer (10mM Tris, 1mM EDTA, pH 7.5). The DNA was electroporated using a Bio-Rad Gene Pulser (Bio-Rad Laboratories) into the Acc-098 cells using the technique of Hensley *et al.* in *J. Biol. Chem.* 269, 23949-23958 (1994). 1.2×10^7 cells were washed once in 12.5 ml of ice cold PBSucrose (PBS, 272mM sucrose, 7mM sodium phosphate pH 7.4, 1mM $MgCl_2$), resuspended in 0.8 ml of PBS, added to 50ul of the DNA solution and incubated on ice for 15 min. The cells were pulsed at 380 V and 25 microfarads, then incubated on ice for 10 min. Cells were plated into 96 well culture plates at 5×10^5 cells/plate in maintenance medium for 24 hr prior to selection. Cells were selected for resistance to 400ug/ml G418 (Geneticin, Life Technologies, Inc.) in maintenance medium. 24 hr prior to assay, the cells were fed with 150ul of the maintenance medium.

Conditioned medium from individual colonies was assayed using an electrochemiluminescence (ECL) detection method on an Origen analyzer (IGEN, Inc.). See Yang *et al.*, *Biotechnology*, 12, 193-194 (1994).

All solutions necessary for the performance of the assays (assay buffer) and for the operation of the analyzer (cell cleaner) were obtained from IGEN. The antibodies (anti-human IgG (g-chain specific), Sigma Chemicals and $F(ab')_2$ Fragment to Human IgG (H+L), Kirkegaard & Perry Laboratories Inc.) were labelled with TAG-NHS-ester (IGEN, Inc.) at a 7:1 molar ratio of TAG:protein, while the Protein A (Sigma) was labelled with Biotin-LC-Sulfo-NHS-ester (IGEN, Inc.) at a 20:1 molar ratio Biotin:protein, both according to IGEN's recommendations. Streptavidin-coated magnetic beads (M-280) were obtained from Dynal.

Immunoassays were performed using the following protocol: per sample, 50ul of the Streptavidin-coated

beads (final concentration 600 ug/ml diluted in PBS, pH7.8, with 1.25% Tween) were mixed with 50ul Biotin-Protein A (final concentration 1ug/diluted in PBS, pH7.8, with 1.25% Tween) and incubated at room
5 temperature for 15min with agitation, 50ul of the TAG antibodies (a mixture with a final concentration of 1.25 ug/ml F(ab')₂ Fragment to Human IgG (H+L) and 0.25 ug/ml Anti-Human IgG (g-chain specific) diluted in PBS, pH7.8, with 1.25% Tween) were added, the solution was then
10 added to 50ul of conditioned medium and incubated with agitation at room temperature for 1 hr. 200ul of assay buffer was added to the reaction mix and the sample analyzed on the Origen I analyzer to measure ECL. The results indicated that approximately 20-37% of the
15 colonies assayed secrete over 15 ng/ml of the antibody with an average expression of about 150 ng/ml.

Humanized anti-Factor IX mAbs were purified from the conditioned media using a Procep A capture step followed by ion-exchange chromatography to reduce the
20 DNA burden. Procep A sorbent material (Bioprocessing Ltd., Durham, England) was used to prepare a column with a 1:1 diameter to height ratio. Clarified conditioned media was loaded onto the column at about 150 cm/hr. The column was washed sequentially with phosphate
25 buffered saline (PBS), PBS containing 1 M NaCl, and finally with PBS. The bound material was recovered with 0.1 M acetic acid elution. The eluate was adjusted to pH 5.5 and was diluted (1:4) with water. The diluted solution was loaded onto an S-Sepharose column (2.5 x 13
30 cm) which was pre-equilibrated with 20 mM sodium acetate, pH 5.5 at 80 cm/hr. The column was washed with the acetate buffer until a steady baseline was obtained and the bound protein was eluted with 20 mM sodium phosphate, pH 7.4 at 25 cm/hr. The eluted material was
35 filtered with a 0.4 micron membrane and stored at 4°C.

Example 7**Mouse-Human Chimeric Antibody**

100 ng of BC2 RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions
5 (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming, and PCR amplified with synthetic *ScaI* (SEQ ID NO: 100) and *NarI* (SEQ ID NO: 101) primers to produce the BC2 light chain variable region with *ScaI*, *NarI* ends (SEQ ID NOS: 102 and 103). This DNA was ligated into
10 *ScaI*, *NarI* digested F9HZHC1-3 (SEQ ID 77) and digested with *ScaI*, *NarI* to produce a mouse-human chimeric light chain F9CHLC (SEQ ID NOS: 104 and 105).

100 ng of BC2 RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions
15 (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming, and PCR amplified with synthetic *SpeI* (SEQ ID NO: 106) and *NheI* (SEQ ID NO: 107) primers to produce the BC2 heavy chain variable region with *SpeI*, *NheI* ends (SEQ ID NOS: 108 and 109). The campath signal sequence
20 was PCR amplified from the RSVHZ19 heavy chain (SEQ ID NO: 25) with *EcoRI* (SEQ ID 26) and *SpeI* (SEQ ID 87) primers. These two DNA fragments were ligated into a *EcoRI*, *NheI* digested IL4CHHCpcd vector described in published International Patent Application No.
25 WO95/07301, replacing the IL4 variable region with the BC2 Factor IX mouse variable region, to produce a mouse-human chimeric heavy chain F9CHHC (SEQ ID Nos: 110 and 111).

Co-transfection and purification of the mouse-human
30 chimeric antibody ch α FIX was accomplished as described above for the humanized constructs.

Example 8**Efficacy of humanized Factor IX mAbs in Rat Thrombus****Model**

In order to evaluate the efficacy of humanized
5 anti-Factor IX antibodies in prevention of arterial
thrombosis, the rat carotid artery thrombosis model as
described above in Example 3 was used. Baseline
parameters were established for carotid blood flow,
arterial pressure, heart rate, vessel patency and
10 activated partial thromboplastin time (aPTT). Fifteen
minutes thereafter, carotid injury was effected for 10
minutes. The parameters were determined 60 minutes
after onset of carotid injury. Carotid thrombus was
also extracted from the carotid artery and weighed.

15 All agents were administered intravenously 15
minutes before the onset of carotid injury. The
following treatments were examined and compared to the
anti-Factor IX mAb BC2.

1. Vehicle
- 20 2. ch α FIX: 3 mg/kg bolus
3. SB 249413: 3 mg/kg bolus
4. SB 249415: 3 mg/kg bolus
5. SB 249416: 3 mg/kg bolus
6. SB 249417: 3 mg/kg bolus
- 25 7. SB 257731: 3 mg/kg bolus
8. Heparin: 60 units/kg bolus + 2 units/kg/min
infusion

The aPTT was used as the primary criterion for
30 evaluation of efficacy versus bleeding liabilities of
the anti-coagulant/thrombotic agents used in the study.
The results in Fig. 8 demonstrate that the humanized
Factor IX mAbs SB 249413, SB 249415, SB 249416, SB
249417 and SB 257731 had a modest effect on aPTT at 3.0
35 mg/kg which is within the clinical accepted range.

The effect of the Factor IX mAbs on thrombus mass is shown in Fig. 9. The results indicate that all of the humanized mAbs are equally effective in reducing thrombus mass.

5 The studies conducted in the rat carotid thrombosis model clearly demonstrate the efficacy of the humanized Factor IX mAbs in prevention of thrombosis in a highly thrombogenic arterial injury model. Most notably, the efficacy of all of the humanized Factor IX mAbs was
10 demonstrated within the desired therapeutic anticoagulant target defined by the aPTT.

Example 9

Antibody Biochemical and Biophysical Properties

15 The molecular mass of SB 249417 was determined by MALD-MS to be 148,000Da. Analytical ultracentrifugation of SB 249417 gave an identical value. In the presence of Factor IX plus Ca^{2+} , the antibodies derived from BC 2 sedimented with a mass of 248,000Da corresponding to the
20 combined mass of the mAb and two molecules of Factor IX. No evidence of higher ordered aggregates was observed in the presence or absence of Factor IX.

The kinetics of Factor IX binding to SB 249417 was assessed by BIAcore analysis with antibody bound to an
25 immobilized protein A surface. Recombinant human Factor IX (rhFIX, Genetics Institute) at 49 nM was used and measurements performed in the presence of 5 mM Ca^{2+} . The interaction was characterized by rapid association,
30 $k_{\text{ass}} = 2.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and relatively slow off-rate, $k_{\text{diss}} = 4.1 \times 10^{-4} \text{ s}^{-1}$. The calculated K_{d} for Factor IX binding was 1.9 nM.

Table 1 summarizes the biophysical properties of SB 249417.

Table 1

Summary of the Biophysical Properties of SB 249417

5	Isotype	IgG1, kappa
	Purity by SDS-PAGE	>95% (under reducing conditions)
	Molecular Weight	
10	Mass Spectrometry	148,000 Da
	Analytical Ultracentrifugation	148,000 Da
	Stoichiometry of Factor IX Binding	
	Isothermal Titration Calorimetry	1.5 moles Factor IX: 1 mole mAb
15		
	Factor IX Binding Affinity	
	Isothermal Titration Calorimetry	Kd= 4 nM at 25°C
	Biosensor	Kd= 2 nM
20		
	Factor IX Binding Kinetics	
	Biosensor	$k_{ass} = 2.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ $k_{diss} = 4 \times 10^{-4} \text{ s}^{-1}$
25	Table 2 summarizes the factor IX binding properties of mAbs of the present invention. The calculated dissociation constants were essentially identical within experimental error.	

Table 2

Kinetics of Factor IX Binding to Anti-Factor IX mAbs

<u>mAb</u>	<u>k_{ass} ($M^{-1}s^{-1}$)</u>	<u>k_{diss} (s^{-1})</u>	<u>calc. K_D (nM)</u>
SB 249417	2.0×10^5	4.1×10^{-4}	1.9
BC2	4.8×10^5	9.1×10^{-4}	1.9
Chf9	2.4×10^5	3.0×10^{-4}	1.3
SB 249413	6.5×10^5	2.8×10^{-3}	3.7-5.1
SB 249415	7.5×10^5	1.8×10^{-4}	1.1-2.3
SB 249416	5.2×10^5	4.1×10^{-4}	0.8
SB 257731	9.2×10^5	9.9×10^{-4}	1.1
SB 257732	1.1×10^6	1.2×10^{-3}	1.5

5

The interactions between rhFIX and SB 249417 , BC2 and other humanized constructs were characterized by titration microcalorimetry, which measures binding interactions in solution from the intrinsic heat of binding. Nine injections of 106 uM FIX were made into the calorimeter containing 2 uM mAb SB 249417. Binding was detected in the first 4 injections as exothermic heats. At the last 5 injections the mAb binding sites were saturated with FIX and only background heats of mixing were observed. The results indicated that the equivalence point occurred at a molar binding ratio near 2 FIX per mAb, as expected. Nonlinear least squares analysis of the data yield the binding affinity.

The rhFIX affinities of the mAbs were measured over a range of temperature from 34-44°C in 10mM HEPES, 10mM CaCl₂, 150mM NaCl, pH 7.4. These data allow the affinity at 37°C to be determined directly and the affinity at 25°C to be calculated from the van't Hoff

equation. The data in Table 3 indicate that the affinities of SB 249417, BC2 and its other humanized constructs are within error (a factor of 2) the same.

Table 3

5 **Titration Calorimetry Results for Anti-FIX mAbs**

mAb	Kd, nM at 25°C	Kd, nM at 37°C	Molar Binding Ratio FIX/mAb
BC2	10	20	1.4
SB 249413	6	12	1.9
SB 249415	3	7	1.7
SB 249417	4	12	1.5
SB 257732	4	9	1.8

The mAbs SB 249413, SB 249415, SB 249417 and SB 257732 all exhibited very similar thermal stabilities by differential scanning calorimetry. Their unfolding Tms ranged from 70-75°C indicating high stability against thermally induced denaturation.

10

Example 10

15 **Mechanism of Antibody-Mediated Inhibition of Factor IX**

A library of chimeric constructs composed of sequences of Factor IX spliced into the framework of the homologous protein Factor VII was constructed and used to map the epitope for the Factor IX BC2 mAb. See Cheung *et al.*, *Thromb. Res.* 80, 419-427 (1995). Binding was measured using a BiaCore 2000 surface plasmon

20

resonance device. The BC2 antibody was coupled directly to the chip using the NHS/EDC reaction. Binding was measured by 2 min of contact time at 20uL/min with 200 nM of each of the given constructs in 25 mM MOPS, pH 7.4, 0.15 M NaCl, 5 mM CaCl₂. Dissociation was monitored for 3 min using the same buffer with no protein. No binding was detected to the wild type construct in the presence of 50 mM EDTA. The data are presented in Table 4.

10

Table 4

Summary of Binding of Factor IX Constructs to BC2 Antibody

Construct	Degree of Binding
Plasma IXa	Binds
r-IX	Binds
Plasma VII	No Binding
IX LC/VII HC	Binds
IX-A/VII	Binds
VII gla/IX	No Binding
VII-A/IX	No Binding
VII gla (IX 3-11)/IX	Binds
VII gla (IX 3-6)/IX	Very Low Binding
VII gla (IX 9-11)/IX	Very Low Binding
IX K5A	Binds

15

These data indicate that the constructs containing the Factor IX light chain and Factor VII heavy chain (IX LC/VII HC); the Factor IX gla and aromatic stack domains (IX-A/VII); residues 3-11 of Factor IX gla domain within

the Factor VII gla domain (VII gla (IX 3-11)/IX); and Factor IX having a lysine to alanine substitution at residue 5 (IX K5A) exhibit binding to BC2. The VII gla (IX 3-11)/IX construct exhibited BC2 binding equivalent to wild type Factor IX (plasma IXa and r-IX). Thus, the BC2 antibody binds to an epitope contained within residues 3-11 of the Factor IX gla domain.

Example 11

10 Treatment of Arterial Thrombosis with Anti-Factor IX Antibody and Tissue Plasminogen Activator

Administration of tPA with or without adjunctive therapies, was initiated following complete occlusion of the carotid artery. Blood flow in the artery was continuously monitored.

15 Male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 300-490 gm were anesthetized with sodium pentobarbital (55 mg/kg, i.p.). The rats were placed dorsal on a heated (37°C) surgical board and an incision was made in the neck; the trachea was isolated and cannulated with a PE-240, Intramedic tube. The left carotid artery and jugular vein were then isolated. A Parafilm M sheet (4 mm², American National Can) was placed under the carotid artery and an electromagnetic blood flow probe (Carolina Medical) was placed on the artery to measure blood flow. A cannula (Tygon, 0.02" x 0.04", Norton Performance Plastics) was inserted into the jugular vein for drug administration. The left femoral artery was then isolated and cannulated for measurement of blood pressure and collection of blood samples.

Thrombosis in the carotid artery was initiated with a 6.5 mm diameter circular patch of glass micro-filter paper saturated with FeCl₃ solution (50%) placed on the carotid artery downstream from the flow probe for 10 minutes as described in Example 3. In this well-

characterized model, thrombus formation is usually complete within 15 min.

The anti-Factor IX antibody, SB 249415 was administered as a bolus in combination with tPA (Genentech, South San Francisco, CA), while heparin (Elkins-Sinn Inc., Cherry Hill, NJ) was administered as a bolus followed by infusion. All drug infusions continued to the end of the experimental period - 60 minutes from the time of vessel occlusion. Blood samples, 1 mL, were collected for aPTT and PT assay at 0, 30 and 60 min (end of study) from the femoral artery into 3.8% citrate solution and centrifuged. aPTT and prothrombin time (PT) were monitored by a fibrometer (BB1L, Baxter Dade or MLA Electra 800 Automatic Coagulation Timer) with standard procedures. At the end of the experiment, the thrombus was extracted from the carotid artery and weighed.

All data are presented as mean group values \pm SEM for the indicated number of rats in each group. ANOVA and Bonferoni tests for multiple comparisons were used for between group analyses and a $p < 0.05$ accepted as significance.

Formation of an occlusive thrombus occurs approximately 15 min after initiation of arterial injury by application of the FeCl₃ treated patch to the rat carotid artery. As shown in Fig. 10, with tPA alone, reperfusion of the occluded vessel was only observed following administration of a dose of 9 mg/kg tPA with 67% of the treated vessels exhibiting regain of blood flow during the 60 min protocol. At this dose of tPA inclusion of 60 U/kg heparin or 3 mg/kg anti-Factor IX antibody, SB 249415, did not result in a further increase in the incidence of reperfusion suggesting that in the FeCl₃ injury model about 30% of the thrombi are refractory to lysis.

The results in Fig. 10 indicate that, at lower doses of tPA, the incidence of reperfusion is significantly dependent upon which anticoagulant was co-administered with the thrombolytic. When 60 U/kg heparin was administered the percentage of vessels showing reperfusion decreased dramatically with only 12.5% and 40% reperfusion observed with 3 and 6 mg/kg tPA, respectively. Co-administration of 3 mg/kg SB 249415 with the tPA, however, achieved greater than 60% reperfusion with 3 mg/kg tPA and 79% reperfusion with the 6 mg/kg tPA dose. Thus, the anti-Factor IX antibody significantly shifts the thrombolytic dose response curve allowing reperfusion with lower doses of thrombolytic agent.

Thrombolysis and clot formation are dynamic processes and periods of patency followed by re-occlusion were sometimes observed. Since carotid blood flow was monitored continuously during the 60 min experimental protocol, it was also possible to quantitate the total time of carotid patency. As shown in Fig. 11, the total period of vessel patency is substantially increased by combination of 3 mg/kg anti-Factor IX antibody plus tPA. This is particularly evident at the lowest and the intermediate doses of tPA, 3 and 6 mg/kg, respectively. At a combined dose of 3 mg/kg SB 249415 plus 3 mg/kg tPA, the total patency time was 30.6 ± 9.2 min compared to 7.1 ± 7.1 min for the combination of 60 U/kg heparin plus 3 mg/kg tPA. Patency time was zero with 3 mg/kg tPA alone. With a dose of 6 mg/kg tPA, co-administration with heparin increases patency time only slightly to 12.9 ± 6.0 min whereas the tPA-SB 249415 combination achieves maximal patency time of 38.7 ± 8.4 min. Only at the highest dose of tPA (9 mg/kg) does the heparin combination approach the patency achieved with SB 249415, 31.9 ± 4.8 min and 38.0 ± 8.4 min, respectively.

Rapid restoration of blood flow following arterial infarct is critical to minimizing damage to the ischemic tissue. The results in Fig. 12 indicate that the combination of anti-factor IX antibody with tPA resulted in decreased time to reperfusion compared to tPA alone or heparin plus tPA and that this is achieved with lower doses of tPA. When thrombolysis was effected with 3 mg/kg SB 249415 plus 3 mg/kg tPA the time to thrombolysis was 29.4 ± 9.2 min. With 3 mg/kg tPA, alone, no reperfusion was observed. With 60 U/kg heparin plus 3 mg/kg tPA the time to thrombolysis was 52.8 ± 7.1 min. At higher doses of tPA, 6 and 9 mg/kg, the antibody plus tPA treatment regimen achieved initial thrombolysis in 19.4 ± 6.3 and 20.8 ± 8.7 min, respectively. In the absence of added anticoagulant, the time to thrombolysis was 60 min (i.e., the limit of the experimental protocol) and 27.5 ± 6.4 min for doses of 6 and 9 mg/kg, respectively. With addition of 60 U/kg heparin, the corresponding times to thrombolysis were 44.0 ± 7.1 and 27.0 ± 4.9 min. Thus, earlier reperfusion was always achieved with SB 249415 than with heparin or with tPA alone.

Example 12

Effect of Anti-Factor IX Antibody on Hemostatic Function

The impact of anti-factor IX or heparin as adjunctive agents on the maintenance of hemostatic function was determined by monitoring levels of fibrinogen, plasminogen and alpha-2-antiplasmin at the end of the treatment period in rats treated with tPA alone, tPA plus heparin and tPA plus SB 249415 and the results were compared to vehicle treated animals. As shown in Fig. 13, increasing doses of tPA resulted in decreased levels of each of the hemostatic markers measured. Alpha-2-antiplasmin levels dropped from about 90% in animals not treated with tPA to about 20% as the

dose of tPA was increased to 9 mg/kg. Plasminogen levels dropped from an average of about 100% without tPA treatment to about 40% in the 9 mg/kg treatment group. Likewise, fibrinogen levels dropped from about 150 mg/dL to about 90 mg/dL in the high-dose tPA group. Interestingly, the selection of the adjunctive agent does not appear to significantly effect any of these markers. At each tPA dose, similar levels of alpha-2-antiplasmin, plasminogen and fibrinogen were observed in animals given vehicle, 30 or 60 U/kg heparin or 1 or 3 mg/kg SB 249415; the observed decrease in the hemostatic markers is only a function of dose of the thrombolytic agent, tPA, and these decreases, especially in the case of fibrinogen, appear to be particularly large with tPA doses greater than 6 mg/kg, i.e., in the 9 mg/kg high-dose group.

The effects of the different treatment regimens on the standard aPTT coagulation assay aPTT was also monitored (Fig. 14). With increasing doses of tPA the aPTT increased from 19.3s \pm 0.6s to 30.0s \pm 1.6s for vehicle and 9 mg/kg tPA, respectively. Administration of 3 mg/kg SB 249415 produced a limited increase in the aPTT of control animals to 49.6 s \pm 6.4 s. When SB 249415 was co-administered with tPA the observed increase was slightly larger and was dependent upon the dose of tPA. Combination of SB 249415 with 3 mg/kg tPA produced an aPTT of 58.3 s \pm 5.2 s whereas combination of SB 249415 with the 9 mg/kg dose of tPA increased the aPTT to 77.3 s \pm 19.7 s. Administration of either the 30 U/kg or 60 U/kg dose of heparin resulted in large increases in the aPTT. Without tPA the aPTT ranged from about 300 s to 600 s for 30 and 60 U/kg doses, respectively. In tPA treated animals the aPTT was about 800 s.

The elevation of the aPTT obtained with heparin, particularly when coupled with the perturbation of

hemostatic parameters due to the need for high doses of tPA to achieve effective reperfusion, is likely to contribute to bleeding liabilities. Conversely, SB 249415 does not cause major elevation of the aPTT and enables the use of lower doses of tPA providing significant advantage in thrombolytic therapy in myocardial infarction and stroke.

Example 13

10 SB 249417 Enhances the Lytic Potential of Tissue Plasminogen Activator in Stroke

The rat thromboembolic stroke model utilized in these studies was essentially as described by Busch et al. in *Brain Research*, 778, 16-24 (1997). The three principal steps of the model are preparation of emboli, preparation of the rat followed by embolization, and pharmacologic intervention.

Whole blood was withdrawn from a donor rat into a citrated vacutainer tube. The citrated blood (500ul) was promptly added to a test tube containing 1 unit of human thrombin and 5ul of 1M CaCl₂ for a final CaCl₂ concentration of 10 mM. Within 5-10 seconds, a small portion of this cocktail was drawn into an ~15cm length of PE50 catheter and allowed to clot at room temperature for 1 hour. At the end of the period, the tubular clot was extruded from the catheter into a saline-filled petri dish and cut into 1.5 mm length sections. Twelve of these sections (clots) were transferred to a solution of saline containing 0.04mg/ml rat albumin and were drawn back into a PE50 catheter in a volume of ~60ul. The clots which were lined up head to tail in the catheter for embolization into the rat.

Male Sprague Dawley rats weighing 350-400g were surgically prepared to receive a subcutaneous dose of atropine (0.5mg/kg) and were then anesthetized with 5% isoflurane followed by a maintenance dose of 2%. The

body temperature was kept between 37-38°C. Under aseptic conditions, a sagittal midline incision was made in the cervical area, exposing the right common carotid artery (CCA), right internal carotid artery (ICA), right external carotid artery (ECA), and the pterygopalatine artery. The pterygopalatine artery was tied off. A length of the ECA was isolated then tied off and cut. The CCA and ICA were clamped and the PE50 catheter containing the emboli was inserted into the ECA stump and advanced to the bifurcation. The ICA clamp was removed and the emboli were slowly infused into the ICA while simultaneously unclamping the CCA. Infusion of either vehicle (saline), SB 249417 (2.0mg/kg) and/or t-PA (5.0mg/kg) was begun intravenously through a caudal vein 5 minutes post-embolization. SB 249417 was infused as a single bolus dose, whereas the t-PA dose of 5mg/kg was infused as a 10% bolus followed by the remaining 90% over 30 minutes. The surgical incision was closed and the rat was allowed to recover.

Twenty four hours post-embolization, rats were anesthetized and killed. The brain was removed and seven transverse cerebral sections were taken every 2mm from the frontal cerebral pole. The sections were incubated in 1% 2,3,5-triphenyltetrazolium chloride for 20 minutes followed by formalin fixation. The stained cerebral sections were photographed and analyzed using an image analysis system (Optimus Inc., Bothell, Wash). The area of infarction in mm² was calculated by tracing the infarction on the computer screen by a blinded operator. The aggregate mean infarct for each treatment is shown in Fig. 15 (control (n=20), tPA (n=7) and SB 249417 (n=6)). Dosing rats post-embolus with tPA (5.0mg/kg) caused a reduction of ~33% in the resultant mean infarct volume. Administration of SB 249417 alone caused an ~70% reduction in the resulting infarct volumes. The combination of tPA (5.0mg/kg) and SB

249417 (2.0mg/kg) provided further protection, resulting in a reduction of ~88% in mean infarct volumes (P = 0.126). Infarcts in this model occurred with a similar frequency in the striatum and neocortex. Based upon the location of the infarcted tissue, the most frequent site of occlusion appeared to be the middle cerebral artery (MCA). Although less frequent, the evidence suggested that the choroidal, anterior and posterior cerebral arteries were occasionally occluded as well. When viewed by coronal section (data not shown), the majority of infarct volume reduction occurring on treatment was within the central MCA perfusion territory. The distribution of infarcted tissue did not change appreciably after the treatments.

The results indicate that an anti-Factor IX antibody such as SB 249417 when administered post-embolus can reduce the formation of infarcted brain tissue when used as a monotherapy or as an adjunct to a thrombolytic agent such as tPA. Anti-Factor IX antibodies such as SB 249417 are expected to have clinical utility in the treatment of thromboembolic stroke either alone or as an adjunct to thrombolytic agents. Combination therapy would allow for a reduction in the amount of thrombolytic agent and a subsequent reduction in the risk of promoting hemorrhagic stroke.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof, and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indicating the scope of the invention.

CLAIMS

1. A method for treating an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment.

2. The method of claim 1 wherein the anti-Factor IX antibody or antibody fragment is administered post-embolus.

3. The method of claim 1 wherein the anti-Factor IX antibody or antibody fragment is administered post-stroke.

4. The method of claim 1 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249413, SB 249415, SB 249416, SB 249417, SB 257731 or SB 257732.

5. The method of claim 4 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249417.

6. A method for treating an animal post-thromboembolic induced ischemia comprising administering SB 249417.

7. A method for treating an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment in combination with a plasminogen activator.

8. The method of claim 7 wherein the anti-Factor IX antibody or antibody fragment and plasminogen activator are administered post-embolus.

9. The method of claim 7 wherein the anti-Factor IX antibody or antibody fragment and plasminogen activator are administered post-stroke.

10. The method of claim 7 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249413, SB 249415, SB 249416, SB 249417, SB 257731 or SB 257732.

11. The method of claim 10 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249417.

12. The method of claim 7 wherein the thrombolytic agent is tPA, urokinase, streptokinase or variants thereof.

13. The method of claim 12 wherein the thrombolytic agent is tPA.

14. A method for treating an animal post-thromboembolic induced ischemia comprising administering SB 249417 in combination with tPA.

15. A method for reducing a required dose of a thrombolytic agent in treatment of an animal post-thromboembolic induced ischemia comprising administering an anti-Factor IX antibody or antibody fragment in combination with the thrombolytic agent.

16. The method of claim 15 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249413, SB 249415, SB 249416, SB 249417, SB 257731 or SB 257732.

17. The method of claim 16 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249417.

18. The method of claim 15 wherein the thrombolytic agent is tPA, urokinase, streptokinase or variants thereof.

19. The method of claim 18 wherein the thrombolytic agent is tPA.

20. A method for preventing thromboembolic stroke in an animal comprising administering an anti-Factor IX antibody or antibody fragment to an animal at risk for thromboembolic stroke.

21. The method of claim 20 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249413, SB 249415, SB 249416, SB 249417, SB 257731 or SB 257732.

22. The method of claim 20 wherein the anti-Factor IX antibody or antibody fragment has the identifying characteristics of SB 249417.

23. A method of preventing thromboembolic stroke in an animal comprising administering SB 249417 to an animal at risk for thromboembolic stroke.

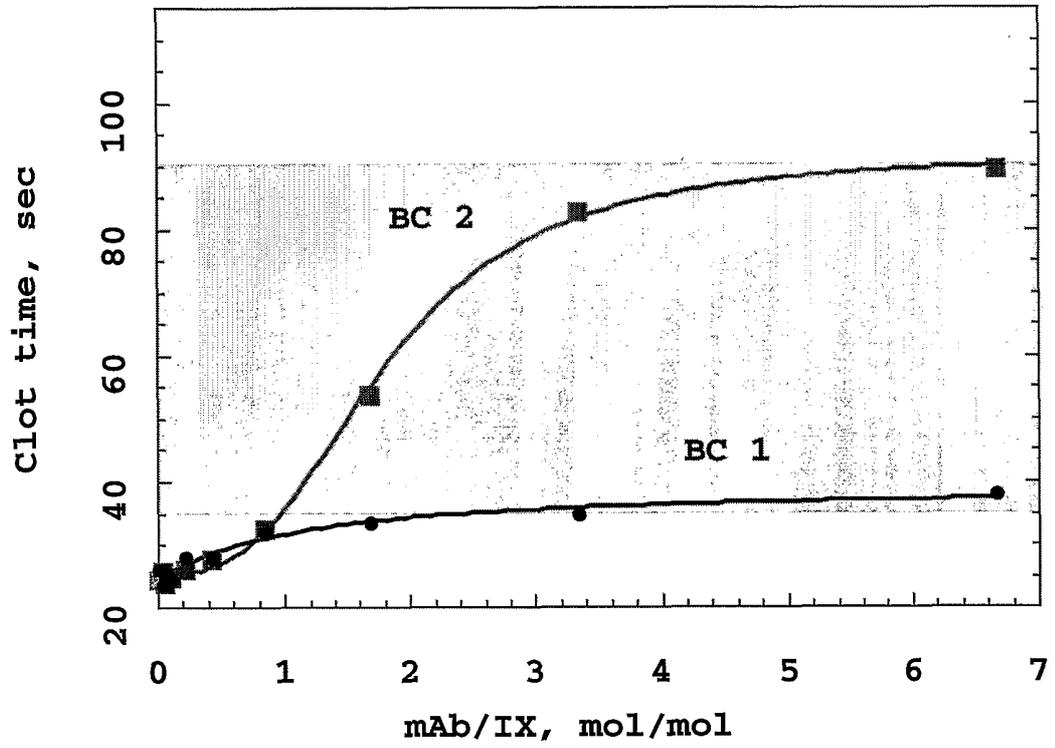


FIGURE 1

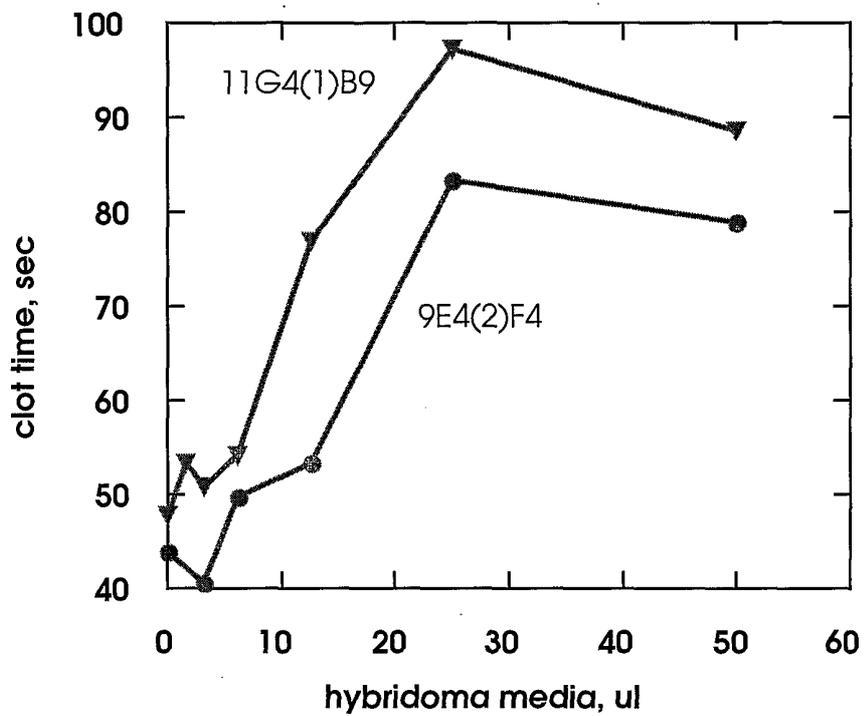


FIGURE 2

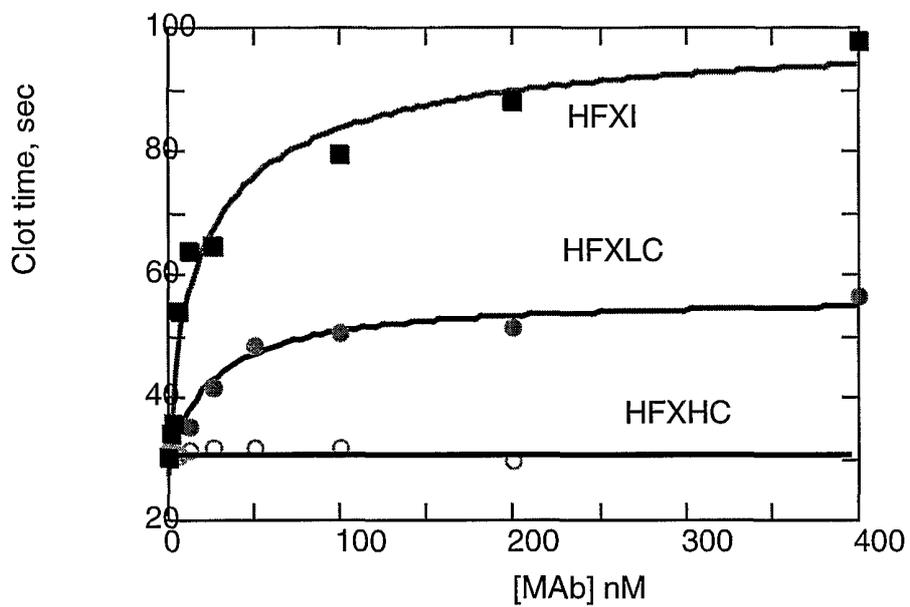


FIGURE 3

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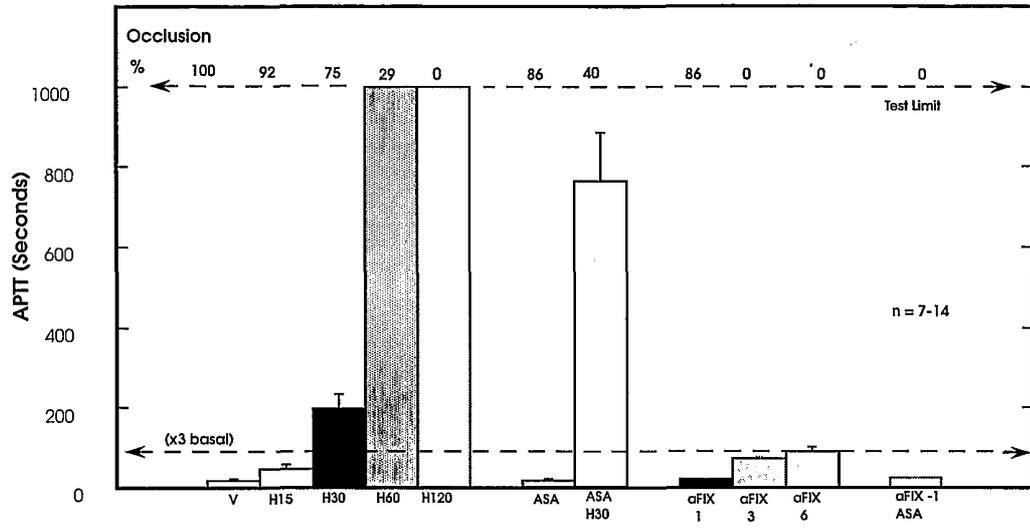


FIGURE 4

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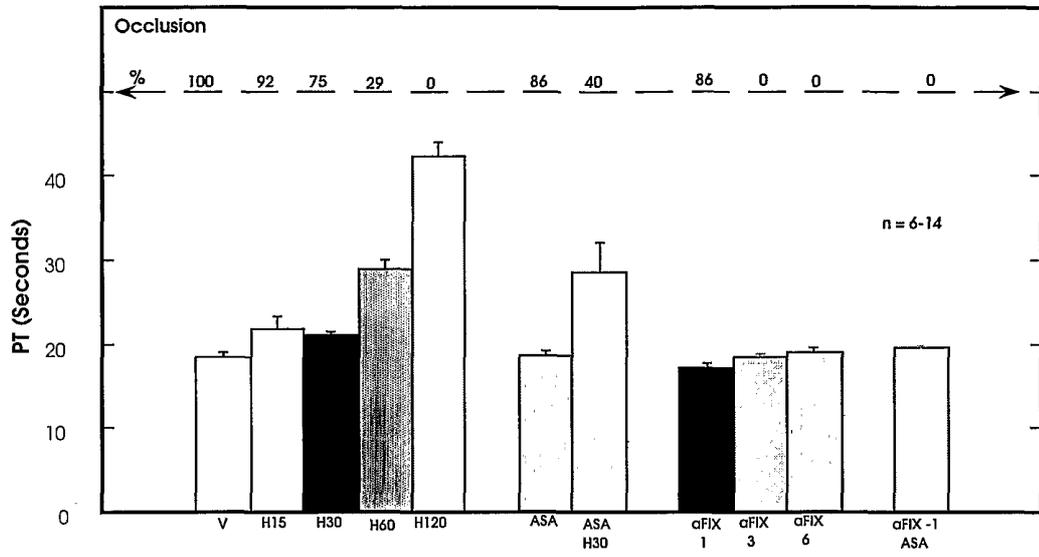


FIGURE 5

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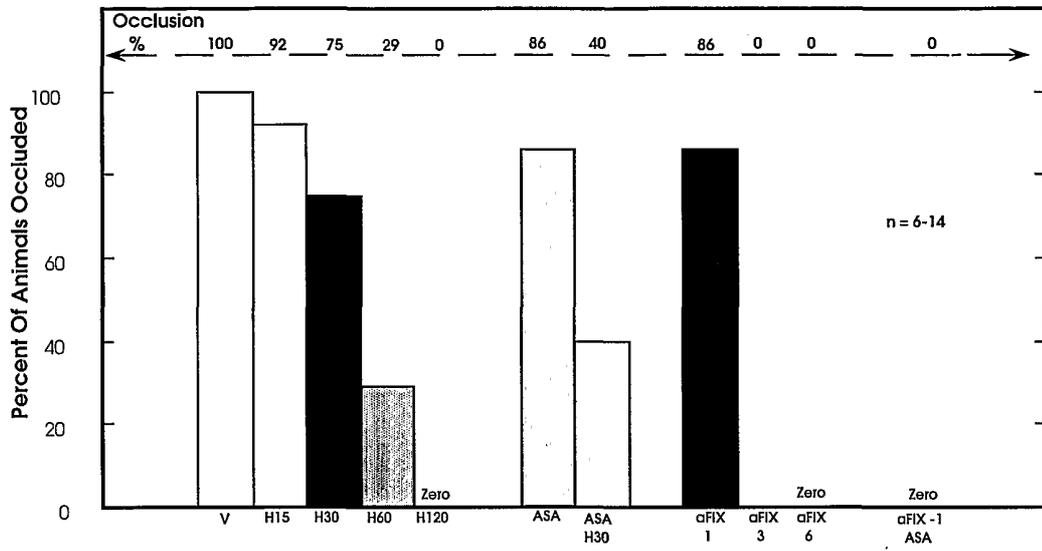


FIGURE 6

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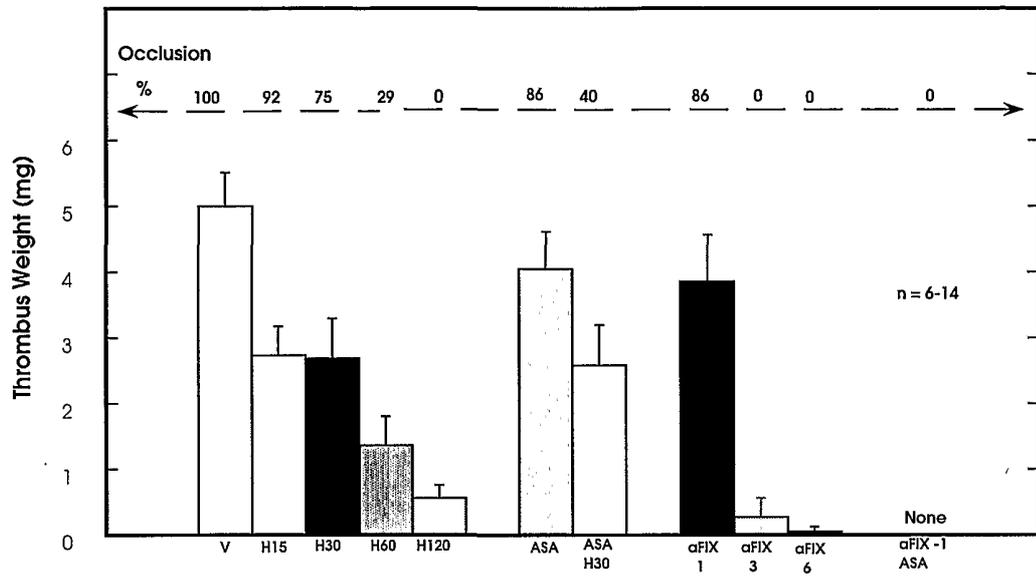


FIGURE 7

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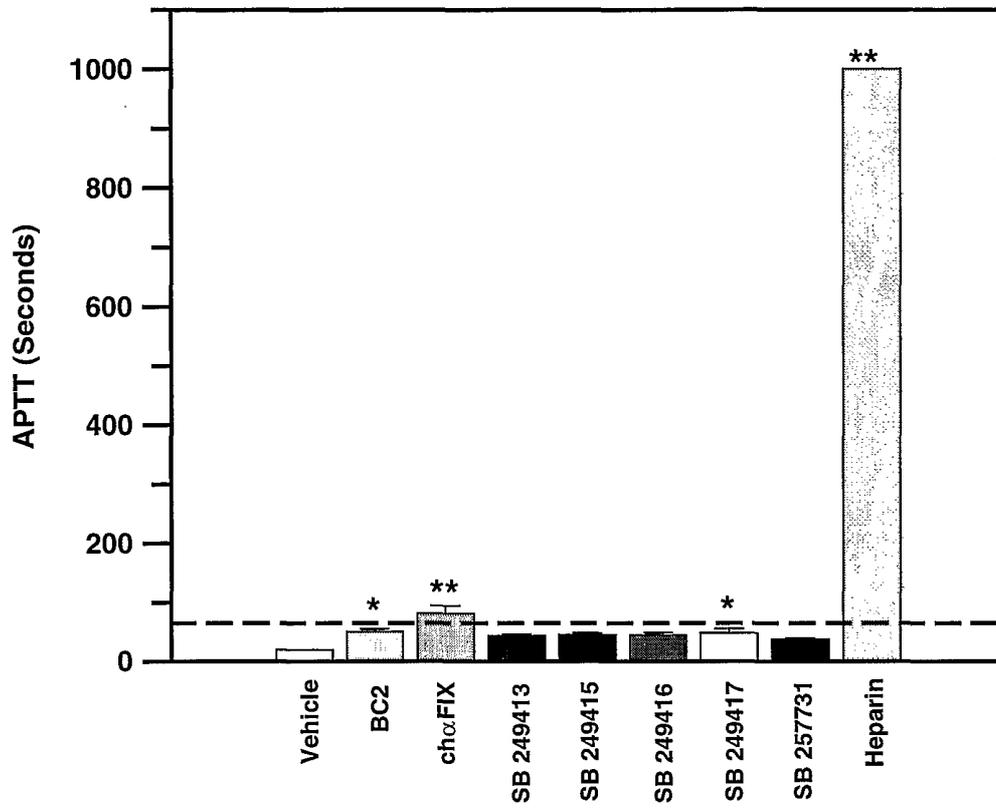


FIGURE 8

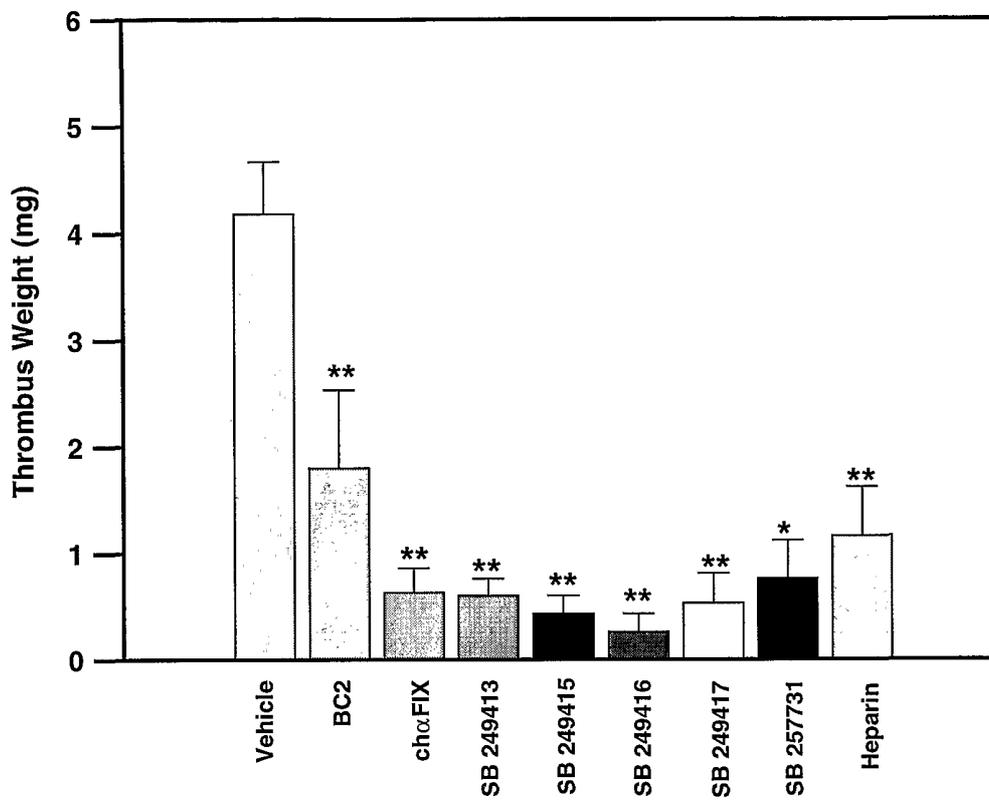


FIGURE 9

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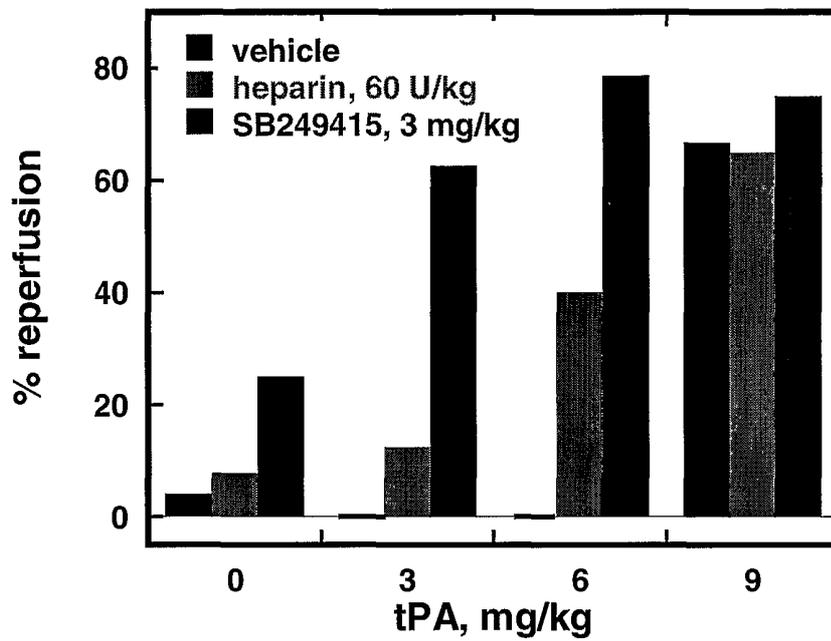


FIGURE 10

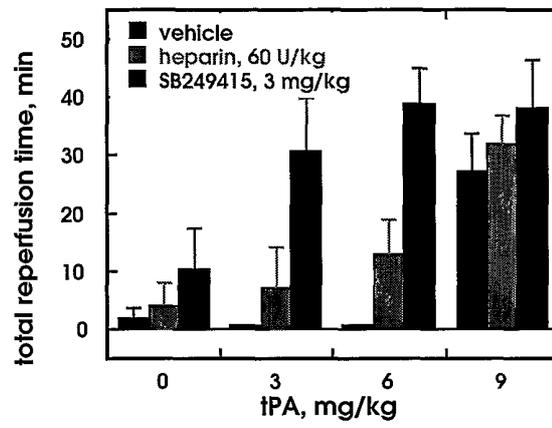


FIGURE 11

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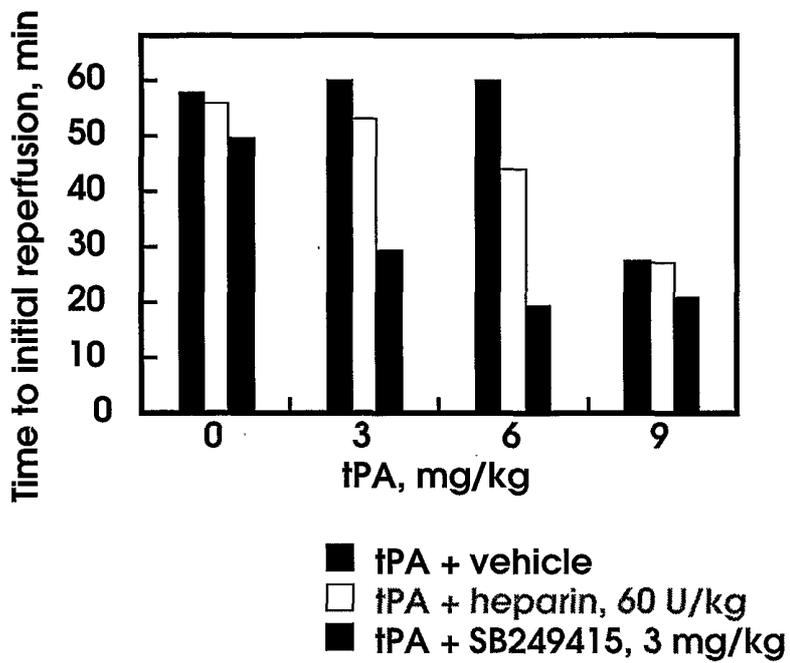


FIGURE 12

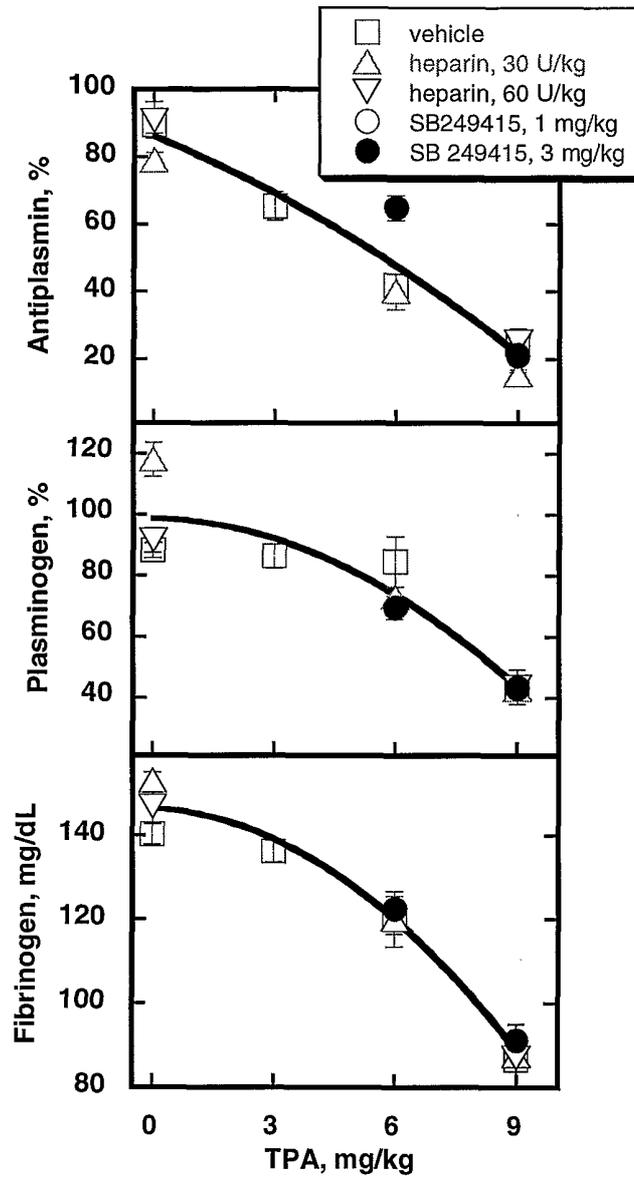
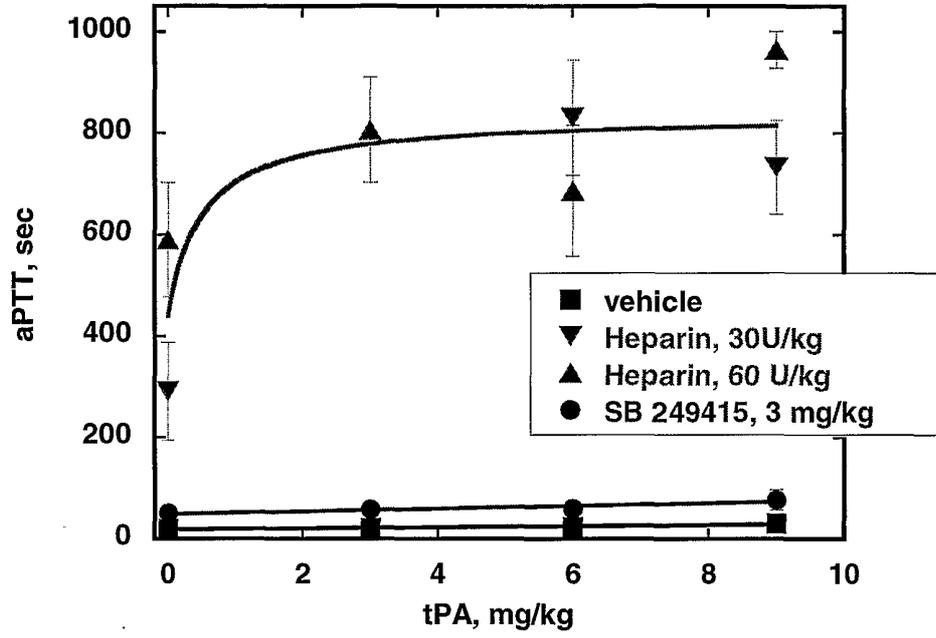
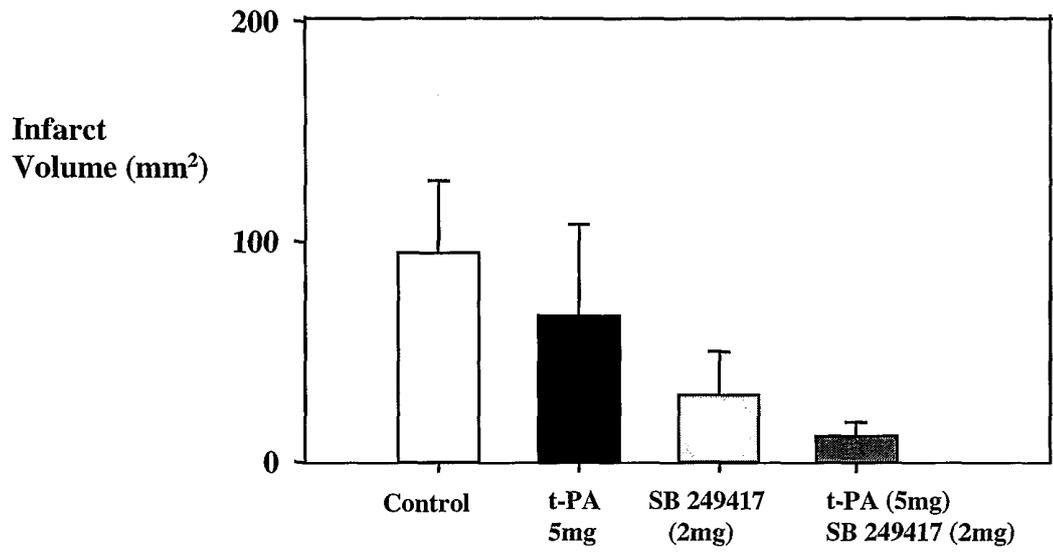


FIGURE 13



15/15



SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: Blackburn, Michael
Feuerstein, Giora
Barone, Frank C.
Toomey, John R.

- (ii) TITLE OF INVENTION: ANTICOAGULANT AGENTS USEFUL IN
TREATMENT OF THROMBOSIS

- (iii) NUMBER OF SEQUENCES: 111

- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: King of Prussia
 - (D) STATE: PA
 - (E) COUNTRY: USA
 - (F) ZIP: 19406

- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ Version 1.5

- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: unknown
 - (B) FILING DATE: herewith
 - (C) CLASSIFICATION:

- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/571,434
 - (B) FILING DATE: 15-MAY-2000

- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Baumeister, Kirk
 - (B) REGISTRATION NUMBER: 33,833
 - (C) REFERENCE/DOCKET NUMBER: P50438-2

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 610-270-5096
- (B) TELEFAX: 610-270-5090
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 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:

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

CATCCTAGAG TCACCGAGGA

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGCTGCCCAA AGTGCCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTAACACTCA TTCCTGTTGA AGCTCTTGAC AATGGG

36

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATTTTCARG TGCAGATTTT C

21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CAGATCCAGT TGGTGCAGTC TGGACCTGAG CTGAAGAAGC CTGGAGAGAC AGTCAAGATC      60
TCCTGCAAGG CTTCTGGGTA CACCTTCACA AACTATGGAA TGAAGTGGGT GAAGCAGGCT      120
CCAGGAAAGG GTTTAAAGTG GATGGGCTGG ATAAACACCA GAAATGGAAA GTCAACATAT      180
GTTGATGACT TCAAGGGACG GTTTGCCTTC TCTTTGGAAA GCTCTGCCAG CACTGCCAAT      240
TTGCAGATCG ACAACCTCAA AGATGAGGAC ACGGCTACAT ATTTCTGTAC AAGAGAAGGG      300
AATATGGATG GTTACTTCCC TTTTACTTAC TGGGGCCAAG GGACTCTGGT CACTGTCTCT      360
GCA                                                                                   363
    
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

CAAATTGTTC TCTCCCAGTC TCCAGCAATC CTGTCTGCAT CTCCAGGGGA GAAGGTCACA      60
ATGACTTGCA GGGCCAGCTC AAGTGTAAT TACATGCACT GGTACCAGCA GAAGCCAGGA      120
TCCTCCCCCA AACCTGGAT TTATGCCACA TCCAACCTGG CTTCTGGAGT CCCTGCTCGC      180
TTCAGTGGCA GTGGGTCTGG GACCTCTTAC TCTCTCACAA TCAGCAGAGT GGAGGCTGAA      240
GATGCTGCCA CTTATTACTG CCAGCAGTGG AGTATTAACC CACGGACGTT CGGTGGAGGC      300
ACCAAGCTGG AAATCAAACG G                                                                                   321
    
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

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

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asn Tyr Gly Met Asn

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
CAACTAGTGC AATCTGGGTC TGAGTTGAAG AAGCCTGGGG CCTCAGTGAA GGTTTCCTGC      60
AAGGCCTCTG GATACACCTT CACTAACTAT GGAATGAACT GGGT                          104
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
TTGAAGTCAT CAACATATGT TGACTTTCCA TTTCTGGTGT TTATCCATCC CATCCACTCG      60
AGCCCTTGTC CAGGGGCCTG TCGCACCCAG TTCATTCCAT AGTTAGTG                          108
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTCAACATAT GTTGATGACT TCAAGGGGCG GTTTGTCTTC CCTCTGTCAG CACGGCATAT	60
CTACAGATCA GCAGCCTAAA GGCTGACGAC ACTGCAGTGT ATTACTG	107

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 91 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGTACCCTGG CCCAGTAAG TAAAAGGGAA GTAACCATCC ATATTCCCTT CTCTCGCACA	60
GTAATACACT GCAGTGTTCGT CAGCCTTTAG G	91

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 337 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 2...337
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

A	CTA	GTG	CAA	TCT	GGG	TCT	GAG	TTG	AAG	AAG	CCT	GGG	GCC	TCA	GTG	AAG	49
	Leu	Val	Gln	Ser	Gly	Ser	Glu	Leu	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	
	1				5					10					15		
	GTT	TCC	TGC	AAG	GCC	TCT	GGA	TAC	ACC	TTC	ACT	AAC	TAT	GGA	ATG	AAC	97
	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Gly	Met	Asn	
			20						25					30			
	TGG	GTG	CGA	CAG	GCC	CCT	GGA	CAA	GGG	CTC	GAG	TGG	ATG	GGA	TGG	ATA	145
	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Trp	Ile	
			35				40						45				
	AAC	ACC	AGA	AAT	GGA	AAG	TCA	ACA	TAT	GTT	GAT	GAC	TTC	AAG	GGG	CGG	193
	Asn	Thr	Arg	Asn	Gly	Lys	Ser	Thr	Tyr	Val	Asp	Asp	Phe	Lys	Gly	Arg	
			50				55					60					
	TTT	GTC	TTC	TCC	TTG	GAC	ACC	TCT	GTC	AGC	ACG	GCA	TAT	CTA	CAG	ATC	241
	Phe	Val	Phe	Ser	Leu	Asp	Thr	Ser	Val	Ser	Thr	Ala	Tyr	Leu	Gln	Ile	
	65					70					75				80		
	AGC	AGC	CTA	AAG	GCT	GAC	GAC	ACT	GCA	GTG	TAT	TAC	TGT	GCG	AGA	GAA	289
	Ser	Ser	Leu	Lys	Ala	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Glu	
					85					90				95			
	GGG	AAT	ATG	GAT	GGT	TAC	TTC	CCT	TTT	ACT	TAC	TGG	GGC	CAG	GGT	ACC	337
	Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr	Tyr	Trp	Gly	Gln	Gly	Thr	
					100					105				110			

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 112 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

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

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCTACTAGTG CAATCTGGGT CTGAGTTGAA GCC

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGGGTACCCT GCGCCAGTA AGTAAAAGGG

30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...95

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC

53

Met Gly Trp Ser Cys Ile Ile Leu Phe

1

5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG GTC CAA CTA GT
 Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu
 10 15 20

97

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Gln Val Gln Leu
 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 110 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGAGACGCCA	TCGAATTCTG	AGCACACAGG	ACCTCACCAT	GGGATGGAGC	TGTATCATCC	60
TCTTCTTGGT	AGCAACAGCT	ACAGGTGTCC	ACTCCCAGGT	CCAACTGCAG		110

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGAGACGCCA	TCGAATTCTG	A	21
------------	------------	---	----

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GATTGCACTA	GTTGGAĆCTG	GGAGTGGACA	30
------------	------------	------------	----

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTAGAGTGGG TCGCAGAGAT CTCTGATGGT GGTAGTTACA CCTACTATCC AGACACTGTG 60
 ACGGGCCGGT TCACGAT 77

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ATCGTGAACC GGCCCGTCAC AGTGTCTGGA TAGTAGGTGT AACTACCACC ATCAGAGATC 60
 TCTGCGACCC ACT 73

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...363
- (D) OTHER INFORMATION: F9HZHC 1-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAG GTG CAA CTA GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC	48
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala	
1 5 10 15	
TCA GTG AAG GTT TCC TGC AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT	96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr	
20 25 30	
GGA ATG AAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG	144
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met	
35 40 45	
GGA TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC	192
Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe	
50 55 60	
AAG GGA CGG TTT GTC TTC TCC TTG GAC ACC TCT GTC AGC ACG GCA TAT	240
Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr	
65 70 75 80	
CTA CAG ATC AGC AGC CTA AAG GCT GAC GAC ACT GCA GTG TAT TAC TGT	288
Leu Gln Ile Ser Ser Leu Lys Ala Asp Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
GCG AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC	336
Ala Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly	
100 105 110	

CAG GGT ACC CTG GTC ACC GTC TCC TCA
 Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

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

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 165 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```

AGTACTGACA CAGTCTCCAG CCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC      60
CTGCAGGGCC AGCTCAAGTG TAAATTACAT GCACTGGTAC CAACAGAGAC CTGGCCAGGC      120
TCCCAGGCTC CTCATCTATG CCACTAGTAA CCTGGCTTCT GGCAT                        165
    
```

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 146 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

CCGCGGGTTA ATACTCCACT GCTGACAGTA ATAAACCGCA AAATCTTCAG GCTCTAGACT      60
GCTGATGGTG AGAGTGAAAT CTGTCCCAGA CCCGGATCCA CTGAACCTGG CTGGGATGCC      120
AGAAGCCAGG TTACTAGTGG CATAGA                                             146
    
```

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 280 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 2...280
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

A	GTA	CTG	ACA	CAG	TCT	CCA	GCC	ACC	CTG	TCT	TTG	TCT	CCA	GGG	GAA	AGA	49
	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly	Glu	Arg	
	1			5					10					15			
	GCC	ACC	CTC	TCC	TGC	AGG	GCC	AGC	TCA	AGT	GTA	AAT	TAC	ATG	CAC	TGG	97
	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met	His	Trp	
			20						25					30			
	TAC	CAA	CAG	AGA	CCT	GGC	CAG	GCT	CCC	AGG	CTC	CTC	ATC	TAT	GCC	ACT	145
	Tyr	Gln	Gln	Arg	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr	Ala	Thr	
			35					40						45			
	AGT	AAC	CTG	GCT	TCT	GGC	ATC	CCA	GCC	AGG	TTC	AGT	GGA	TCC	GGG	TCT	193
	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	
			50					55						60			
	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	AGT	CTA	GAG	CCT	GAA	GAT	TTT	241
	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu	Asp	Phe	
	65					70					75					80	
	GCG	GTT	TAT	TAC	TGT	CAG	CAG	TGG	AGT	ATT	AAC	CCG	CGG				280
	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg				
					85						90						

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 93 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```

Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1             5             10             15
Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp
          20             25             30
Tyr Gln Gln Arg Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Thr
          35             40             45
Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser
          50             55             60
Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe
65             70             75             80
Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg
          85             90
    
```

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TCGAGTACTG ACACAGTCTC CAGCCAC

27

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACCGCGGGT TAATACTCCA CTGCTGA

27

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 94 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...92

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC

53

Met Gly Trp Ser Cys Ile Ile Leu Phe

1

5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAG ATA GTA CT

94

Leu Val Ala Thr Ala Thr Gly Val His Ser Glu Ile Val

10

15

20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Glu Ile Val
                20
    
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACTGTGTCA GTACTATCTC GGAGTGGACA

30

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGGCAGCCTC CTAAGTTGCT CATTACTGG GCGTCGACTA GGAATCTGG GGTAC

55

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 51 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CCCAGATTCC CTAGTCGACG CCCAGTAAAT GAGCAACTTA GGAGGCTGCC C

51

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 321 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...321
- (D) OTHER INFORMATION: F9HZLC1-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GAA	ATA	GTA	CTG	ACA	CAG	TCT	CCA	GCC	ACC	CTG	TCT	TTG	TCT	CCA	GGG	48
Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly	
1				5					10					15		
GAA	AGA	GCC	ACC	CTC	TCC	TGC	AGG	GCC	AGC	TCA	AGT	GTA	AAT	TAC	ATG	96
Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met	
			20					25					30			
CAC	TGG	TAC	CAA	CAG	AGA	CCT	GGC	CAG	GCT	CCC	AGG	CTC	CTC	ATC	TAT	144
His	Trp	Tyr	Gln	Gln	Arg	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr	
		35					40					45				
GCC	ACT	AGT	AAC	CTG	GCT	TCT	GGC	ATC	CCA	GCC	AGG	TTC	AGT	GGA	TCC	192
Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser	
	50					55					60					
GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	AGT	CTA	GAG	CCT	GAA	240
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu	
65					70					75				80		
GAT	TTT	GCG	GTT	TAT	TAC	TGT	CAG	CAG	TGG	AGT	ATT	AAC	CCG	CGG	ACG	288
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg	Thr	
				85					90				95			
TTC	GGC	GGA	GGG	ACC	AAG	GTG	GAG	ATC	AAA	CGA						321
Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg						
			100					105								

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 107 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```

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

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```

CCTGGACAAG GGCTCAAGTG GATGGGATGG ATAAACACCA GAAATGGAAA GTCAACATAT      60
GTTGATGACT TCAAGGGACG GTTTGTCTTC TCTCTAGACT CCTCTGTCAG CACGGCATAT      120
CTACAGATCA GCAG                                     134
    
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
GGTACCCTGG CCCAGTAAG TAAAAGGGAA GTAACCATCC ATATTCCTT CTCTCGTACA    60
GTAATACACT GCAGTGTCGT CAGCCTTTAG GCTGCTGATC TGTAGATATG CCGTGCTGAC    120
AGAGGAGTCT AGAG                                                    134
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 225 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...225

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
CCT GGA CAA GGG CTC AAG TGG ATG GGA TGG ATA AAC ACC AGA AAT GGA    48
Pro Gly Gln Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Arg Asn Gly
1           5           10           15
```

AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC TCT CTA 96
 Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe Ser Leu
 20 25 30

GAC TCC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA AAG GCT 144
 Asp Ser Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala
 35 40 45

GAC GAC ACT GCA GTG TAT TAC TGT ACG AGA GAA GGG AAT ATG GAT GGT 192
 Asp Asp Thr Ala Val Tyr Tyr Cys Thr Arg Glu Gly Asn Met Asp Gly
 50 55 60

TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC 225
 Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr
 65 70 75

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

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

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 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:

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTTCCTGGAC AAGGGCTCAA GTGGATG

27

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 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:

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TTTGGTACCC TGGCCCCAGT AAGT

24

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 363 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...363
- (D) OTHER INFORMATION: F9HZHC 1-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CAG GTG CAA CTA GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC	48
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala	
1 5 10 15	
TCA GTG AAG GTT TCC TGC AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT	96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr	
20 25 30	
GGA ATG AAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTC AAG TGG ATG	144
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met	
35 40 45	
GGA TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC	192
Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe	
50 55 60	
AAG GGA CGG TTT GTC TTC TCT CTA GAC TCC TCT GTC AGC ACG GCA TAT	240
Lys Gly Arg Phe Val Phe Ser Leu Asp Ser Ser Val Ser Thr Ala Tyr	
65 70 75 80	
CTA CAG ATC AGC AGC CTA AAG GCT GAC GAC ACT GCA GTG TAT TAC TGT	288
Leu Gln Ile Ser Ser Leu Lys Ala Asp Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
ACG AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC	336
Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly	
100 105 110	
CAG GGT ACC CTG GTC ACC GTC TCC TCA	363
Gln Gly Thr Leu Val Thr Val Ser Ser	
115 120	

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

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

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 82 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CAACAGAGAC CTGGCCAGGC TCCAAGCCC TGGATCTATG CCACGAGTAA CCTGGCTAGC	60
GGCGTCCCAG CCAGGTTTCAG TG	82

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 90 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GATCCACTGA ACCTGGCTGG GACGCCGCTA GCCAGGTAC TCGTGGCATA GATCCAGGGC	60
TTGGGAGCCT GGCCAGGTCT CTGTTGGTAC	90

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr Ala Thr Ser
 1 5 10 15
 Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
 20 25

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...321

(D) OTHER INFORMATION: F9HZLC 1-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GAA ATA GTA CTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG 48
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 GAA AGA GCC ACC CTC TCC TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG 96
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Asn Tyr Met
 20 25 30
 CAC TGG TAC CAA CAG AGA CCT GGC CAG GCT CCC AAG CCC TGG ATC TAT 144
 His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr
 35 40 45
 GCC ACG AGT AAC CTG GCT AGC GGC GTC CCA GCC AGG TTC AGT GGA TCC 192
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

GGG TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTA GAG CCT GAA 240
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

GAT TTT GCG GTT TAT TAC TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG 288
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr
 85 90 95

TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CGA 321
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

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

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GATCCGGGTC TGGGACAGAT TACACTCTCA CGATATCCAG T

41

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CTAGACTGGA TATCGTGAGA GTGTAATCTG TCCCAGACCC G

41

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide


```

GCC ACG AGT AAC CTG GCT AGC GGC GTC CCA GCC AGG TTC AGT GGA TCC      192
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
      50                55                60

GGG TCT GGG ACA GAT TAC ACT CTC ACG ATA TCC AGT CTA GAG CCT GAA      240
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
      65                70                75                80

GAT TTT GCG GTT TAT TAC TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG      288
Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr
                85                90                95

TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CGA                          321
Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
                100                105
    
```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```

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

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 165 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```

AGTACTCACC CAGAGCCCAA GCAGCCTGAG CGCCAGCGTG GGTGACAGAG TGACCATCAC      60
CTGCAGGGCC AGCTCAAGTG TAAATTACAT GCACTGGTAC CAGCAGAAGC CAGGTAAGGC      120
TCCAAAGCCT TGGATCTACG CCACTAGTAA CCTGGCTTCT GGTGT                          165
    
```

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 161 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

```

CCGCGGGTTA ATACTCCACT GCTGGCAGTA GTAGGTGGCG ATATCCTCTG GCTGGAGGCT      60
GCTGATGGTG AAGGTGTAGT CTGTACCGCT ACCGGATCCG CTGAATCTGC TTGGCACACC      120
AGAAGCCAGG TTACTAGTGG CGTAGATCCA AGGCTTTGGA G                          161
    
```

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 280 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 2...280

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

A	GTA	CTC	ACC	CAG	AGC	CCA	AGC	AGC	CTG	AGC	GCC	AGC	GTG	GGT	GAC	AGA	49
	Val	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	
	1				5						10					15	
	GTG	ACC	ATC	ACC	TGC	AGG	GCC	AGC	TCA	AGT	GTA	AAT	TAC	ATG	CAC	TGG	97
	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met	His	Trp	
				20							25					30	
	TAC	CAG	CAG	AAG	CCA	GGT	AAG	GCT	CCA	AAG	CCT	TGG	ATC	TAC	GCC	ACT	145
	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Thr	
				35					40							45	
	AGT	AAC	CTG	GCT	TCT	GGT	GTG	CCA	AGC	AGA	TTC	AGC	GGA	TCC	GGT	AGC	193
	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	
				50				55					60				
	GGT	ACA	GAC	TAC	ACC	TTC	ACC	ATC	AGC	AGC	CTC	CAG	CCA	GAG	GAT	ATC	241
	Gly	Thr	Asp	Tyr	Thr	Phe	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Ile	
	65					70						75				80	
	GCC	ACC	TAC	TAC	TGC	CAG	CAG	TGG	AGT	ATT	AAC	CCG	CGG				280
	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg				
					85							90					

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 93 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```

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

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TTTAGTACTC ACCCAGAGCC CAAGCAG

27

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TTCCGCGGGT TAATACTCCA CTGCTGG

27

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTCGAGCAGT ACTATCTGGG AGTGGACACC TGT

33

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CGCCGCCACA GTCCGTTTGA TTTCCACCTT GGTCCCTTGG CCGAACGTCC GC 52

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 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:

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...321
- (D) OTHER INFORMATION: F9HZLC 2-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CAG ATA GTA CTC ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT 48
 Gln Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

GAC AGA GTG ACC ATC ACC TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG 96
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met
 20 25 30

CAC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CCT TGG ATC TAC 144
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr
 35 40 45

GCC ACT AGT AAC CTG GCT TCT GGT GTG CCA AGC AGA TTC AGC GGA TCC 192
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser

50	55	60	
GGT AGC GGT ACA GAC TAC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG			240
Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu			
65	70	75	80
GAT ATC GCC ACC TAC TAC TGC CAG CAG TGG AGT ATT AAC CCG CGG ACG			288
Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr			
	85	90	95
TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGG			321
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg			
	100	105	

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

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

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Gln Ile Val Leu
                20
    
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 401 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...401

(D) OTHER INFORMATION: F9HZLC 1-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC      53
                Met Gly Trp Ser Cys Ile Ile Leu Phe
                1           5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATA GTA CTG ACA CAG      101
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Val Leu Thr Gln
10           15           20           25

TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC      149
Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser
                30           35           40

TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG TAC CAA CAG AGA      197
    
```

Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met	His	Trp	Tyr	Gln	Gln	Arg			
			45					50					55					
CCT	GGC	CAG	GCT	CCC	AAG	CCC	TGG	ATC	TAT	GCC	ACG	AGT	AAC	CTG	GCT			245
Pro	Gly	Gln	Ala	Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Thr	Ser	Asn	Leu	Ala			
		60					65					70						
AGC	GGC	GTC	CCA	GCC	AGG	TTC	AGT	GGA	TCC	GGG	TCT	GGG	ACA	GAT	TAC			293
Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr			
		75				80						85						
ACT	CTC	ACG	ATA	TCC	AGT	CTA	GAG	CCT	GAA	GAT	TTT	GCG	GTT	TAT	TAC			341
Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr			
		90			95					100					105			
TGT	CAG	CAG	TGG	AGT	ATT	AAC	CCG	CGG	ACG	TTC	GGC	GGA	GGG	ACC	AAG			389
Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys			
					110					115				120				
GTG	GAG	ATC	AAA															401
Val	Glu	Ile	Lys															
																		125

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	Gly			
1				5					10					15				
Val	His	Ser	Gln	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu			
			20					25					30					
Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Ser	Ser	Val			

	35		40		45														
Asn	Tyr	Met	His	Trp	Tyr	Gln	Gln	Arg	Pro	Gly	Gln	Ala	Pro	Lys	Pro				
	50					55					60								
Trp	Ile	Tyr	Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	Phe				
65					70					75					80				
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu				
			85						90					95					
Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn				
			100						105					110					
Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys							
		115					120							125					

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

AGGCCTCTGG ATACACCTTC ACTAACTATG GAATGAACTG GGTGCGACAG GCCCCTGGAC	60
AAGGGCTCGA GTGGATGGGA T	81

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TGTCTAGAGA GAAGACAAAC CGTCCCTTGA AGTCATCAAC ATATGTTGAC TTTCCATTTTC 60
 TGGTGT'TTAT CCATCCCATC CACTCGAGCC CTTGTCCAG 99

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 87 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GGTTTGTCTT CTCTCTAGAC ACCTCTGTCA GCACGGCATA TCTACAGATC AGCAGCCTAA 60
 AGGCTGAGGA CACTGCAGTG TATTTCT 87

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GGTACCCTGG CCCAGTAAG TAAAAGGGAA GTAACCATCC ATATTCCCTT CTCTCGTACA 60
 GAAATACACT GCAGTGTCCCT CAGCCT 86

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 278 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 3...278

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

AG GCC TCT GGA TAC ACC TTC ACT AAC TAT GGA ATG AAC TGG GTG CGA	47
Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg	
1 5 10 15	
CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG GGA TGG ATA AAC ACC AGA	95
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg	
20 25 30	
AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC	143
Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe	
35 40 45	
TCT CTA GAC ACC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA	191
Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu	
50 55 60	
AAG GCT GAG GAC ACT GCA GTG TAT TTC TGT ACG AGA GAA GGG AAT ATG	239
Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met	
65 70 75	
GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC	278
Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr	

80

85

90

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```

Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln
 1             5             10             15
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg Asn
          20             25             30
Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe Ser
          35             40             45
Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys
          50             55             60
Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met Asp
65             70             75             80
Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr
          85             90
    
```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

AGGCCTCTGG ATACACCTTC ACTAACTATG

30

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGTACCCTGG CCCAGTAAG TAAAAG

26

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CCAGACTCGA CTAGTTGGAT CTGGGAGTGG ACACCTG

37

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 446 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:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 27...446
- (D) OTHER INFORMATION: F9HZHC 3-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC	53
Met Gly Trp Ser Cys Ile Ile Leu Phe	
1 5	
TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATC CAA CTA GTG CAA	101
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Gln Leu Val Gln	
10 15 20 25	
TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC	149
Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys	
30 35 40	
AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT GGA ATG AAC TGG GTG CGA	197
Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg	
45 50 55	
CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG GGA TGG ATA AAC ACC AGA	245
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg	
60 65 70	
AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC	293
Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe	
75 80 85	
TCT CTA GAC ACC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA	341

Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu
 90 95 100 105

AAG GCT GAG GAC ACT GCA GTG TAT TTC TGT ACG AGA GAA GGG AAT ATG 389
 Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met
 110 115 120

GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC CTG GTC ACC 437
 Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
 125 130 135

GTC TCC TCT 446
 Val Ser Ser
 140

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

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

	100		105		110										
Tyr	Phe	Cys	Thr	Arg	Glu	Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr
	115				120					125					
Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser				
	130				135					140					

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 90 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

AGTACTGACA CAGTCTCCAT CCTCCCTGTC TGCATCTGTT GGGGACAGAG TCACCATCAC	60
TTGCAGGGCC AGCTCAAGTG TAAATTACAT	90

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CTTGATGGGA CGCCGCTAGC CAGGTTACTC GTGGCATAGA TCCAGGGCTT GGGAGCTTTG	60
CCAGGTTTCT GTTGGTACCA GTGCATGTAA TTTACACTTG AGCTGGCC	108

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

TAACCTGGCT AGCGCGTCC CATCAAGGTT CAGTGGATCC GGGTCTGGGA CAGATTACAC	60
TCTCAGATA TCCAGTCTAC AACCTGAAGA TTTTGC GACT TATTACTG	108

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GGCGCCGCCA CAGTTCGTTT GATCTCCAGC TTGGTCCCTC CGCCGAACGT CCGCGGGTTA	60
ATACTCCACT GCTGACAGTA ATAAGTCGCA AAATCTTCAG GT	102

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 330 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:
- (ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 2...328

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

A	GTA	CTG	ACA	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTT	GGG	GAC	AGA	49
	Val	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	
	1			5					10					15			
GTC	ACC	ATC	ACT	TGC	AGG	GCC	AGC	TCA	AGT	GTA	AAT	TAC	ATG	CAC	TGG		97
Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met	His	Trp		
			20					25					30				
TAC	CAA	CAG	AAA	CCT	GGC	AAA	GCT	CCC	AAG	CCC	TGG	ATC	TAT	GCC	ACG		145
Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Thr		
			35				40						45				
AGT	AAC	CTG	GCT	AGC	GGC	GTC	CCA	TCA	AGG	TTC	AGT	GGA	TCC	GGG	TCT		193
Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser		
			50				55					60					
GGG	ACA	GAT	TAC	ACT	CTC	ACG	ATA	TCC	AGT	CTA	CAA	CCT	GAA	GAT	TTT		241
Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe		
					70					75					80		
GCG	ACT	TAT	TAC	TGT	CAG	CAG	TGG	AGT	ATT	AAC	CCG	CGG	ACG	TTC	GGC		289
Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg	Thr	Phe	Gly		
				85						90				95			
GGA	GGG	ACC	AAG	CTG	GAG	ATC	AAA	CGA	ACT	GTG	GCG	GCG	CC				330
Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala					
			100							105							

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 109 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

```

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

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CAAGTACTGA CACAGTCTCC ATCCTC

26

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

AGGGCGCCGC CACAGTTCGT TTGATC

26

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 412 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...412

(D) OTHER INFORMATION: F9HZLC 3-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC	53
Met Gly Trp Ser Cys Ile Ile Leu Phe	
1 5	
TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATA GTA CTG ACA CAG	101
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Val Leu Thr Gln	
10 15 20 25	
TCT CCA TCC TCC CTG TCT GCA TCT GTT GGG GAC AGA GTC ACC ATC ACT	149
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr	
30 35 40	
TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG TAC CAA CAG AAA	197
Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp Tyr Gln Gln Lys	
45 50 55	
CCT GGC AAA GCT CCC AAG CCC TGG ATC TAT GCC ACG AGT AAC CTG GCT	245
Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala	
60 65 70	
AGC GGC GTC CCA TCA AGG TTC AGT GGA TCC GGG TCT GGG ACA GAT TAC	293
Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr	
75 80 85	
ACT CTC ACG ATA TCC AGT CTA CAA CCT GAA GAT TTT GCG ACT TAT TAC	341
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr	
90 95 100 105	
TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG TTC GGC GGA GGG ACC AAG	389
Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr Phe Gly Gly Gly Thr Lys	
110 115 120	
CTG GAG ATC AAA CGA ACT GTG GC	412
Leu Glu Ile Lys Arg Thr Val Val	
125	

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

```

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

(2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CAAATAGTAC TCTCCCAGTC TCCAGC

26

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GGATAAGCTT GGCGCCGCAA CAGTCGGTTT GATTTCAGC T

41

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 335 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...335

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CAG ATA GTA CTC TCC CAG TCT CCA GCA ATC CTG TCT GCA TCT CCA GGG
 Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly

48

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

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 318 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:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...318

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

CAG ATA GTA CTC TCC CAG TCT CCA GCA ATC CTG TCT GCA TCT CCA GGG 48
 Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
 1 5 10 15
 GAG AAG GTC ACA ATG ACT TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG 96
 Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met
 20 25 30
 CAC TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CCC TGG ATT TAT 144

```

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
      35                               40                               45

GCC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT      192
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
      50                               55                               60

GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC AGA GTG GAG GCT GAA      240
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
      65                               70                               75                               80

GAT GCT GCC ACT TAT TAC TGC CAG CAG TGG AGT ATT AAC CCA CGG ACG      288
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr
      85                               90                               95

TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA                               318
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
      100                               105
    
```

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```

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

65		70		75		80									
Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg	Thr
			85						90					95	
Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys						
			100						105						

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

CAGATCCAAC TAGTGCAGTC TGGACCTGAG 30

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 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:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

TTAAGCTTGC TAGCTGCAGA GACAGTGACC AG 32

(2) INFORMATION FOR SEQ ID NO:108:

ACA AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC 336
 Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly
 100 105 110

CAA GGG ACT CTG GTC ACT GTC TCT GCA GCT AGC 369
 Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser
 115 120

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

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

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:

Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly
 100 105 110

CAA GGG ACT CTG GTC ACT GTC TCT GCA
 Gln Gly Thr Leu Val Thr Val Ser Ala
 115 120

363

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/27438

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 39/40, 39/42, 39/395

US CL :424/130.1, 133.1, 141.1, 145.1, 146.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 141.1, 145.1, 146.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

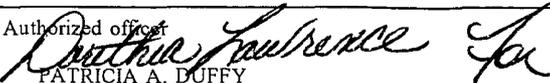
C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	REFINO et al. A Human Antibody that Binds to the gamma-carboxyglutamic Acid Domain of Factor IX is a Potent Antithrombotic In Vivo. Thrombosis and Haemostasis. September 1999, Vol. 82, No. 3, pages 1188-1195 see entire document.	1-6, 20-23 ----- 7-19
X - Y	FEUERSTEIN et al. Antithrombotic Efficacy of a Novel Murine Antihuman Factor IX Antibody in Rats. Atherosclerosis, Thrombosis, and Vascular Biology. October 1999, Vol. 19, No. 10, pages 2554-2562, see entire document.	1-6, 20-23 ----- 7-19

Further documents are listed in the continuation of Box C. See patent family annex.

<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 09 FEBRUARY 2001	Date of mailing of the international search report 03 APR 2001
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  PATRICIA A. DUFFY Telephone No. (703) 308-0196
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/27438

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KESSLER, C. M. The Pharmacology of Aspirin, Heparin, Coumarin, and Thrombolytic Agents, Implications for Therapeutic Use in Cardiopulmonary Disease. Chest. April 1991, Vol. 99, No. 4, pages 97S-112S, see entire document.	7-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/27438

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN-USPATFULL, MEDLINE, BIOSYS, CAB ABSTRACTS, EMBASE, JAPIO.

search terms: Factor IX, antibody, immunoglobulin, thrombosis, PT, PTT, stroke, ischaemia, clot, plasminogen activator