



(72) MAGNANI, JOHN L., US

(71) GLYCOTECH CORPORATION, US

(51) Int.Cl.<sup>6</sup> C12N 5/06, G01N 33/53, A61K 47/48, C12N 5/08, B01D 15/08,  
C12Q 1/04, A61K 49/00

(30) 1997/09/25 (60/059,972) US

(54) **PROCEDES ET COMPOSITIONS UTILISES POUR LIER DES  
CELLULES SOUCHES HEMATOPOIETIQUES**

(54) **METHODS AND COMPOSITIONS FOR BINDING  
HEMATOPOIETIC STEM CELLS**

(57) L'invention concerne des procédés et des compositions utilisés pour lier des cellules souches hématopoïétiques. Les procédés de l'invention consistent généralement à utiliser un partenaire de liaison qui forme un complexe avec une structure à base de lactosamine sialylée présente sur la surface des cellules souches. La formation desdits complexes facilite, par exemple, l'immobilisation, la purification, l'identification et le ciblage de cellules souches hématopoïétiques. Lesdites compositions de l'invention comprennent généralement un partenaire de liaison qui peut être libre, fixé à un matériau de support ou lié à un agent marqueur ou thérapeutique.

(57) Methods and compositions are provided for binding hematopoietic stem cells. The methods generally employ a binding partner that forms a complex with a sialylated lactosamine structure present on the surface of stem cells. The formation of such complexes facilitates, for example, immobilization, purification, identification and targeting of hematopoietic stem cells. The compositions described herein generally comprise a binding partner, which may be free, attached to a support material or linked to a label or therapeutic agent.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 5/06, 5/08, A61K 47/48, 49/00,</b> <b>B01D 15/08, C12Q 1/04, G01N 33/53</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/15628</b> <b>(43) International Publication Date:</b> 1 April 1999 (01.04.99)
<b>(21) International Application Number:</b> PCT/US98/20063 <b>(22) International Filing Date:</b> 24 September 1998 (24.09.98) <b>(30) Priority Data:</b> 60/059,972                      25 September 1997 (25.09.97)      US <b>(71) Applicant (for all designated States except US):</b> GLYCOTECH CORPORATION [US/US]; Suite 200, 14915 Broschart Road, Rockville, MD 20850 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> MAGNANI, John, L. [US/US]; 8115 Runnymede Drive, Frederick, MD 21702 (US). <b>(74) Agents:</b> SHARKEY, Richard, G. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> METHODS AND COMPOSITIONS FOR BINDING HEMATOPOIETIC STEM CELLS		
<b>(57) Abstract</b>  <p>Methods and compositions are provided for binding hematopoietic stem cells. The methods generally employ a binding partner that forms a complex with a sialylated lactosamine structure present on the surface of stem cells. The formation of such complexes facilitates, for example, immobilization, purification, identification and targeting of hematopoietic stem cells. The compositions described herein generally comprise a binding partner, which may be free, attached to a support material or linked to a label or therapeutic agent.</p>		

## METHODS AND COMPOSITIONS FOR BINDING HEMATOPOIETIC STEM CELLS

### TECHNICAL FIELD

The present invention relates generally to methods and compositions for  
5 binding hematopoietic stem cells. The invention is more particularly directed to  
methods for immobilizing, purifying or labeling stem cells. The invention is also  
directed to methods for targeting agents, such as therapeutic agents, to stem cells.

### BACKGROUND OF THE INVENTION

Gene therapy represents a promising approach to the prevention and  
10 treatment of a variety of diseases. In practice, however, the application of genetic  
therapies has been limited by practical problems, such as the inability to target an agent  
specifically to a desired cell type or the limited lifespan of a genetically altered cell.

The use of multipotent hematopoietic progenitor cells as vehicles for  
gene therapy could potentially overcome such difficulties. These stem cells, which are  
15 characterized by a cell surface marker known as CD34, could be genetically modified *in*  
*vitro*, and then transplanted into a patient using routine techniques. Following  
transplantation, the transgenic cells can home directly to bone marrow and, if required,  
fully repopulate and eventually reconstitute all of the blood cells of the host. In this  
fashion, the desired gene product may be produced in a constitutively regenerating  
20 population of cells *in vivo*.

Previous attempts to take advantage of these properties of hematopoietic  
stem cells, however, have been hampered by difficulties associated with *in vitro*  
manipulation. In culture, these progenitor cells lose their pluripotency and differentiate.  
To avoid this problem, stem cells have been co-cultured with microvascular endothelial  
25 feeder cells (primary cells from the brain of non-human sources) and certain cytokines.  
Under such conditions, an expanded population of stem cells has been shown to remain  
CD34<sup>+</sup> and CD38<sup>-</sup> while interacting with feeder cells (*see* Davis et al., *Blood* 85:1751-  
61, 1995). Adhesion to these endothelial cells appears to be a critical requirement for  
maintaining the pluripotent CD34 phenotype. Such co-culture techniques appear to

stabilize the dedifferentiated state of the stem cells sufficiently to allow genetic manipulation. Unfortunately, serious drawbacks (such as the limited lifespan of the feeder cells, potential biohazards and cross-species immune responses) prevent serious consideration of this method for therapeutic use. New methods for binding  
5 hematopoietic stem cells are needed that avoid these drawbacks.

Agents that bind stem cells would also aid in purification of such cells prior to, for example, bone marrow transplantation. Current affinity procedures often involve recognition of an epitope of the CD34 antigen. Within such procedures, problems with specificity and release from the affinity matrix have been encountered.

10 Accordingly, there is a need in the art for improved methods for immobilizing and purifying hematopoietic stem cells that overcome the disadvantages encountered with existing techniques. The present invention fulfills this need and provides further related advantages.

#### SUMMARY OF THE INVENTION

15 Briefly stated, this invention provides methods and compositions for binding hematopoietic stem cells. Within one aspect, the present invention provides methods for immobilizing hematopoietic stem cells, comprising contacting a biological sample containing hematopoietic stem cells with a binding partner, wherein the binding partner binds to a sialylated carbohydrate chain comprising the structure NeuAc $\alpha$ 2-  
20 3Gal $\beta$ 1-4. Within specific embodiments, the binding partner may be attached to a solid support before or after complex formation. Suitable binding partners include antibodies and antigen-binding fragments thereof and lectins. Within further preferred embodiments, the sialylated carbohydrate chain comprises the structure NeuAc $\alpha$ 2-  
25 3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-3)<sub>n</sub>, wherein n is an integer ranging from 1 to 100, and more preferably from 2 to 50.

Within a related aspect, methods for purifying hematopoietic stem cells are provided. Such methods comprise: (a) contacting a biological sample containing hematopoietic stem cells with a binding partner that binds to a sialylated carbohydrate chain comprising the structure NeuAc $\alpha$ 2-3Gal $\beta$ 1-4 to form a stem cell-binding partner  
30 complex; and (b) separating the stem cell-binding partner complex from unbound

biological sample. To facilitate separation, the binding partner may be attached to a solid support before or after complex formation. Suitable binding partners include antibodies and antigen-binding fragments thereof and lectins. Within further preferred embodiments, the sialylated carbohydrate comprises the structure NeuAc $\alpha$ 2-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-3)<sub>n</sub>, wherein n is an integer ranging from 1 to 100, and more preferably from 2 to 50.

Within further aspects, the present invention provides methods for targeting an agent to a hematopoietic stem cell, comprising contacting a biological sample containing hematopoietic stem cells with a binding partner that binds to a sialylated carbohydrate chain comprising the structure NeuAc $\alpha$ 2-3Gal $\beta$ 1-4, wherein the binding partner is associated with an agent. Suitable binding partners include antibodies and antigen-binding fragments thereof and lectins. Within further preferred embodiments, the sialylated carbohydrate chain comprises the structure NeuAc $\alpha$ 2-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-3)<sub>n</sub>, wherein n is an integer ranging from 1 to 100, and more preferably from 2 to 50. Suitable agents include toxic compounds, therapeutic compounds and detectable markers.

The present invention also provides kits for use in the above methods. One such kit, for use in immobilizing hematopoietic stem cells, comprises: (a) a binding partner for stem cells, wherein the binding partner binds to a sialylated carbohydrate chain comprising the structure: NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and (b) a wash solution, wherein the binding partner and wash solution are provided in separate compartments or containers. The binding partner may, but need not, be attached to a support such as a tissue culture dish. Within another embodiment a kit for use in separation and/or purification of stem cells is provided, comprising: (a) a binding partner for stem cells, wherein the binding partner binds to a sialylated carbohydrate chain comprising the structure: NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and (b) a solid support. In a further embodiment, the present invention provides kits for use in labeling or sorting hematopoietic stem cells, comprising (a) a binding partner for stem cells associated with a detectable marker, wherein the binding partner binds to a sialylated carbohydrate chain comprising the structure: NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and (b) a detection reagent,

wherein the binding partner and detection reagent are provided in separate compartments or containers.

Within further embodiments, the present invention provides pharmaceutical compositions, comprising: (a) an agent associated with a binding  
5 partner for stem cells, wherein the binding partner binds to a sialylated carbohydrate chain comprising the structure: NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and (b) a pharmaceutically acceptable carrier.

The compositions of the present invention (and agents therein) are also provided for use as a medicament, and for use for the manufacture of a medicament for  
10 the treatment of a disease, *e.g.*, by gene therapy.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

## 15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph illustrating the binding of monoclonal antibody NUH2 to neoglycoproteins within an ELISA. Four different glycoproteins were used: "O" 3'SL-APEA-BSA; "□" 3'SL-APD-BSA; "◇" 3'SL-BSA; and "×" M6-BSA. Three comprise 3'sialyllactose (3'SL) linked to bovine serum albumin (BSA), either  
20 directly or via 2-(4-aminophenyl)ethylamine (APEA) or aminophenylene diamine (APD). The fourth is M6 linked directly to BSA. The results are presented as OD<sub>450</sub> as a function of neoglycoprotein concentration.

Figure 2 is a histogram illustrating the immobilization of hematopoietic stem cells using representative binding partners. The results are presented as  
25 fluorescence units for each binding partner, as indicated.

Figure 3 is a histogram illustrating the immobilization of hematopoietic stem cells using representative binding partners. The results are presented as fluorescence units for each binding partner, as indicated.

Figure 4 presents the results of two-color FACS analysis comparing the  
30 binding of the representative binding partner NUH2 to surface markers present on

human cord blood cells to the binding observed for the anti-CD34 antibody HPCA2-PE. The data are presented as contour plots which represent the bivariate distribution of green and red fluorescence. Contour lines known as isopleths are drawn to express a cell count at a particular set number.

5 Figure 5 presents the results of two-color FACS analysis comparing the binding of the control IgM antibody to surface markers present on human cord blood cells to the binding observed for the anti-CD34 antibody HPCA2-PE. The data are presented as contour plots which represent the bivariate distribution of green and red fluorescence. Contour lines known as isopleths are drawn to express a cell count at a  
10 particular set number.

#### DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is directed to methods and compositions for binding hematopoietic stem cells. The methods described herein employ a binding partner that binds to a sialylated lactosamine structure on the surface  
15 of such stem cells to form a binding partner-stem cell complex. The formation of such complexes facilitates a variety of manipulations, such as, for example, immobilization, purification, identification and targeting of hematopoietic stem cells. Compositions according to the present invention generally comprise a binding partner, which may be free, attached to a support material or linked to a label or therapeutic agent, depending  
20 on the intended use.

Prior to setting forth the invention in detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. As used herein, the term "binding" refers to a noncovalent association between two separate molecules (each of which may be free (*i.e.*, in solution) or present on the surface of a  
25 cell or a solid support), such that a "complex" is formed. Such a complex may be free or immobilized (either covalently or noncovalently) on a support material. The ability of one molecule to bind to another molecule may generally be evaluated by determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the  
30 component concentrations. In general, two compounds are said to "bind" in the context

of the present invention when the binding constant for complex formation exceeds about  $10^2$  L/mol.

A binding constant may be determined using methods well known to those of ordinary skill in the art. For example, the binding constant for the formation of a complex between a relatively small carbohydrate and a macromolecule binding partner may be determined using equilibrium dialysis. Briefly, two chambers of known volume are separated by a dialysis membrane that allows transfer of the small molecular weight carbohydrate, but not the macromolecule binding partner (*e.g.*, an antibody). The carbohydrate is labeled with a reporter group, such as tritium, and is added to the solution in chamber 1, while the binding partner is placed in the solution in chamber 2. The carbohydrate molecules are then allowed to diffuse into chamber 2 until transfer of the carbohydrate reaches equilibrium (generally about 1 to 3 days). The binding constant may then be determined by measuring the amount of carbohydrate in each chamber (which may be readily determined using the reporter group) and using equation I:

$$K = \frac{[CB]}{[C][B]}$$

I

where [C] is the concentration of uncomplexed carbohydrate, [B] is the concentration of uncomplexed binding partner and [CB] is the concentration of complex. Since [C] is the concentration of carbohydrate in chamber 1 (*i.e.*, [C<sub>1</sub>]); [B] is difference between the original concentration of binding partner (*i.e.*, [B<sub>0</sub>]) and [CB]; and [CB] is the difference between the concentration of carbohydrate in chamber 2 and the concentration of carbohydrate in chamber 1 (*i.e.*, [C<sub>2</sub>]-[C<sub>1</sub>]), equation I may be rewritten in measurable terms as equation II:

$$K = \frac{[C_2][C_1]}{[C_1]([B_0] - ([C_2] - [C_1]))}$$

30

II



NeuAc $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$ 1-Ser-protein

Any agent (*e.g.*, a cell or a molecule) that satisfies the above  
5 requirements may be a binding partner. Within one preferred embodiment, the binding partner comprises an antibody, or a binding fragment thereof, raised against a suitable immunogen. Suitable immunogens include gangliosides adsorbed and coated on acid-treated *Salmonella minnesotae*, human fetal erythrocytes from cord blood or human colonic adenocarcinoma cells.

10 Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). Following one or more injections, the animals are bled periodically.  
15 Polyclonal antibodies specific for the immunogen may then be purified from such antisera by, for example, affinity chromatography using the immunogen coupled to a suitable solid support.

Monoclonal antibodies specific for the carbohydrate structure of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve  
20 the preparation of immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single  
25 colonies are selected and their culture supernatants tested for binding activity against a sialylated carbohydrate as described herein. Hybridomas secreting antibodies having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to  
30 enhance the yield. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified based on their ability



cells coated on acid-treated, *Salmonella minnesotae* (see Nudelman, *J. Biol. Chem.* 264:18719-25, 1989). Antibody NUH2 has been found to bind the two neoglycoproteins recited above and also to branched disialogangliosides containing sialylated lactosamine structures. In two color FACS analysis, antibody NUH2 also  
5 labels all CD34<sup>+</sup> cells identified by the commercially-available antibody HPCA-2-PE.

Within another preferred embodiment, the binding partner comprises a lectin, or a derivative such as an altered or truncated form that retains binding properties, and combinations of lectins and/or derivatives. One suitable truncated form is a complementarity-determining region (CDR) domain (see Saragovic et al., *Science*  
10 253:792-95, 1991). Suitable lectin binding partners include, but are not limited to, plant lectins such as Tomato lectin, *Maackia amurensis* lectin, *Sambucus nigra* lectin, *Triticum vulgare* lectin and *Erythrina cristagalli* lectin; mammalian lectins such as sialoadhesins (see Kelm et al., *Glycoconjugate J.* 13:913-26, 1996) and galectins; and bacterial lectins such as the carbohydrate receptors of *Helicobacter pylori* (see Teneberg  
15 et al., *J. Biol. Chem.* 272:19067-71, 1997).

Binding partners may generally be identified by evaluating the ability of a putative binding partner to bind a test sialylated carbohydrate chain comprising the NeuAc $\alpha$ 2-3Gal $\beta$ 1-4R structure, as described above. Any of a number of well known binding assays may be employed for screening putative binding partners, including  
20 immunoassays. Binding constants for the interaction between binding partner and test carbohydrate may be measured as described above.

Test sialylated carbohydrate chains may generally be prepared from commercially available materials, using methods that will be apparent to those of ordinary skill in the art. For example, lactosamine chains of varying lengths may  
25 generally be prepared according to the method of Srivastava et al., *J. Carbohydrate Chem.* 10:927-933 (1991). Such chains may be sialylated using sialyltransferases or transsialidase enzymes. Sialyltransferases enzymatically transfer sialic acid (NeuAc) from the sugar nucleotide CMP-NeuAc to the acceptor carbohydrate chain. Transsialidases transfer sialic acid between sialylated and non-sialylated carbohydrate  
30 chains. One example is the transfer of NeuAc from 3'sialylactose to lacto-N-neotetraose to produce 3'sialylacto-N-neotetraose as depicted in the neoglycoprotein above.

A "biological sample," as used herein, refers to any tissue sample or preparation of cells that contains hematopoietic stem cells, including blood, bone marrow, buffy coat cells, cord blood and cells that are grown and/or expanded *in vitro*. Such preparations may, but need not, be treated and/or fractionated prior to use within  
5 the methods of the present invention. For example, a biological sample may be a blood preparation, with or without cytokine treatment.

As discussed in further detail below, a binding partner can be used to immobilize stem cells. The term "immobilization" is used herein to refer to a noncovalent interaction between a stem cell and a binding partner, resulting in the  
10 formation of a stem cell-binding partner complex, wherein the binding partner or the complex is attached to a solid support. In one preferred embodiment, the binding partner binds to the stem cell prior to immobilization of the complex. Within another preferred embodiment, the binding partner is attached to the solid support before contact with the stem cells. The term "attach" or "attachment," as used herein, refers to a  
15 covalent or noncovalent interaction between a binding partner or stem cell-binding partner complex and a solid support, such that the binding constant of the interaction is at least  $10^3$  L/mol.

Any of a variety of techniques known to those in the art for attachment to a solid support, and amply described in the patent and scientific literature, may be  
20 employed. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity, or via covalent attachment. Attachment of a binding partner or complex by adsorption may be achieved by contact, in a suitable buffer, with a solid support for a suitable amount of time. The contact time varies with temperature, but is generally between about 5 seconds and 1 day, and typically between about 10  
25 seconds and 2 hours. Attachment by affinity is generally achieved using a support that contains a compound that binds to the binding partner and/or complex (*e.g.*, a support to which avidin is attached may be used to attach a binding partner associated with biotin). Such a compound may itself be attached by adsorption or covalently.

Covalent attachment of a compound to a solid support may be a direct  
30 linkage between a binding partner or complex and functional groups on the support, or may be a linkage by way of a cross-linking agent. Attachment using a cross-linking

agent may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the compound. For example, a binding partner may be bound to a support having an appropriate polymer coating using benzoquinone, by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13) or by condensation of an amino group on the support with a carboxylic acid on the binding partner.

Within some embodiments of the present invention, as discussed below, an agent may be associated with a binding partner such that the agent can be targeted to hematopoietic stem cells. An "agent" may be a compound or a cell, and may be a therapeutic compound, a cytotoxic compound or a label or detectable marker suitable for labeling hematopoietic stem cells (e.g., for cell sorting or *in vivo* imaging). Such association may be noncovalent (such as by way of hydrophobic interactions or incorporation into a liposome), but is preferably covalent and may be achieved by standard recombinant or chemical means. "Targeting," within the present invention, refers to specifically directing an agent to hematopoietic stem cells *in vitro* or *in vivo*, such that the agent is delivered to hematopoietic stem cells more efficiently than to other cell types. For example, a binding agent associated with a detectable marker may be used to identify hematopoietic stem cells within a mixture of cells using standard techniques (e.g., FACS). For successful bone marrow transplantation, however, the population of hematopoietic stem cells need only be enriched several fold and depleted of T cells and other contaminating cells that promote graft vs. host disease.

Within one aspect of the present invention, methods are provided for immobilizing hematopoietic stem cells. A biological sample containing such stem cells is initially contacted with a binding partner. The binding partner may be attached to a solid support, or such attachment can be achieved after complex formation. Preferred solid supports for expansion and gene transduction include tissue culture dishes, hollow fiber bioreactors and bags, such as blood- and cell-collection bags. For purification purposes, chromatography matrices are preferred supports. In general, an excess of

binding partner over the number of stem cells is employed, to facilitate the formation of stem cell-binding partner complexes.

Suitable conditions for contacting the binding partner with the biological sample are generally conditions that favor complex formation and maintain cell  
5 viability. Such conditions may be readily determined by one of ordinary skill in the art by evaluating the binding constant for complex formation at a series of different conditions. Suitable conditions include physiological conditions, such as pH 7.4 isotonic saline (*e.g.*, 0.15M NaCl) at room temperature. It will be evident to those of ordinary skill in the art that the amount of binding partner necessary to achieve adequate  
10 complexation of the stem cells in a given biological sample will depend upon the concentration of stem cells within the sample, the binding constant for the given binding partner and the other materials present in the biological sample. In general a concentration of binding partner ranging from about 0.1  $\mu\text{g/mL}$  to 10  $\text{mg/mL}$ , and typically from about 1  $\mu\text{g/mL}$  to 1  $\text{mg/mL}$ , is sufficient.

15 After complex formation, the stem cell-binding partner complex may be attached to a solid support if necessary. The remainder of the biological sample may then be removed from the immobilized stem cells by any of a variety of known techniques such as, for example, filtration and/or washing with an appropriate buffer.

Hematopoietic stem cells immobilized as described herein may generally  
20 be used for a variety of *in vitro* manipulations, including expansion and gene transduction. The present immobilization procedure generally has the advantage of permitting expansion of stem cells in the dedifferentiated state.

Within a related aspect, the present invention provides methods for purifying hematopoietic stem cells. "Purification" refers to a separation of the stem  
25 cells from at least a portion of the components of the biological sample, and purified stem cells may be present within a stem cell-binding partner complex. Within this aspect, a biological sample is contacted with a binding partner as described above. If the binding partner was not attached to a support prior to contact with the biological sample, the complex is attached after such contact and separated from the unbound  
30 portion of the biological sample. Cells may then be released from the binding partner using any suitable technique. Preferably, release is achieved by incubation with high

concentrations of carbohydrate hapten to compete for binding to the binding partner. Alternatively, cells may be eluted by incubation under conditions that diminish or eliminate the activity of the binding partner (*e.g.*, a change in pH and/or cation concentration). Under appropriate circumstances, cells may be eluted from  
5 immobilized binding partners by mechanical agitation. Optionally, further purification steps may be performed using other affinity materials.

In other aspects, a binding partner as described herein may be used to target an agent to hematopoietic stem cells *in vitro* or *in vivo*. As noted above, any of a variety of agents associated with a binding partner may be directed specifically to  
10 hematopoietic stem cells. For example, an agent may be a detectable marker suitable for cell identification and sorting *in vitro* or imaging *in vivo*. Such markers are well known in the art and include radionuclides, luminescent groups, fluorescent groups, enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, constant immunoglobulin domains and biotin. Alternatively, an agent may be a  
15 therapeutic or cytotoxic compound. Other agents include polynucleotides encoding a detectable marker, therapeutic agent or cytotoxic compound.

To deliver an agent *in vitro* or *ex vivo*, a biological sample is contacted, as described above, with the agent associated with the binding partner. Unbound binding partner is then removed by standard techniques, such as filtration,  
20 centrifugation, precipitation, elution or other appropriate means. For detectable markers, the agent may then be detected by any appropriate method known to those of ordinary skill in the art, the selection of which will depend in part upon the agent used. For example, a fluorescent agent, such as fluorescein, associated with a binding partner may be used for FACS analyses, using well known techniques.

25 For *in vivo* use, an agent associated with a binding partner is typically administered to a subject in the form of a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous suspension or emulsion, which additionally comprises a pharmaceutically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Any  
30 suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Representative carriers include

physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Optionally, a pharmaceutical composition may additionally contain preservatives and/or other additives such as, for example,  
5 antimicrobial agents, anti-oxidants, chelating agents and/or inert gases, and/or other active ingredients. For imaging uses, after passage of sufficient time to allow localization of the detectable marker, the binding partner binds to the hematopoietic stem cells and can be detected using conventional imaging techniques, such as x-ray technologies.

10                 Within further aspects of the present invention, kits for use in any of the methods described herein are provided. Kits for use in immobilizing hematopoietic stem cells generally contain a binding partner, which may be attached to a tissue culture dish. For separation and purification of stem cells, a kit may contain a binding partner in combination with a filter or chromatographic support. Kits for use in labeling, cell  
15 sorting or *in vivo* imaging typically contain a binding partner associated with a detectable marker. For therapeutic use, a kit may contain binding partner associated with a therapeutic agent. In addition to the above components, one or more additional compartments or containers of a kit generally enclose elements, such as reagents, buffers and/or wash solutions, to be used in the method.

20                 The following Examples are offered by way of illustration and not by way of limitation.

## EXAMPLES

Example 1IDENTIFICATION OF SIALYLATED LACTOSAMINE STRUCTURES  
ON HEMATOPOIETIC STEM CELLS

5

This Example demonstrates use of antibody specific for CD34+ cells to identify sialylated lactosamine structures on the surface of hematopoietic stem cells.

Monoclonal antibody NUH2 (ATCC Accession Number HB 9762) was employed within enzyme-linked immunoassays (ELISAs) for binding to carbohydrate structures. Monoclonal antibody NUH2 is an IgM isotype that was developed by immunizing mice with disialogangliosides isolated from colon adenocarcinoma and adsorbed on acid-treated *Salmonella minnesotae* (see U.S. Patent No. 5,227,160).

To generate a neoglycoprotein library, purified oligosaccharides were chemically coupled to human serum albumin. Each neoglycoprotein molecule contained about 10 to 15 chains of one specific purified oligosaccharide. For screening, the neoglycoprotein library was coated onto the surface of the wells in a microtiter plate by incubating the wells filled (100 $\mu$ l) with neoglycoproteins diluted in phosphate-buffered saline, pH 7.4 (PBS) overnight at 4°C. Wells were then blocked by adding 2% bovine serum albumin in PBS and incubating for 2 hours at room temperature. After washing the wells with PBS, monoclonal antibody diluted in PBS at 10 $\mu$ g/ml (100 $\mu$ l) was added to all of the wells. The microtiter plate was incubated at room temperature for 2 hours, after which the plate was washed and the wells were filled (100 $\mu$ l) with peroxidase-labeled goat anti-mouse IgM (KPL labs, Gaithersburg, MD) at a concentration of 1  $\mu$ g/ml and incubated for 1 hour at room temperature. The plate was then washed and 100  $\mu$ l/well of TMB reagent (KPL labs, Gaithersburg, MD) was added. After 5 minutes, 100 $\mu$ l of 1M phosphoric acid was added to each well to stop the color reaction. The intensity of color in each well was then determined by determining the absorbance of light at 450 nm using the Titertek Multiskan MCC/340 (Flow Laboratories, Inc., McLean, VA).

30

As shown in Figure 1, monoclonal antibody NUH2 binds 3'sialyllactose-containing structures.

Example 2IMMOBILIZATION OF STEM CELLS USING A BINDING PARTNER

This Example illustrates the use of an antibody binding partner to  
5 immobilize hematopoietic stem cells.

Immobilization of CD34+ cells to surfaces coated with antibodies and  
lectins was tested by a static cell adhesion assay. Antibodies and lectins diluted in a  
buffer containing 10mM Tris, 0.15M NaCl, 1mM CaCl<sub>2</sub> at pH 7.4 were added to wells  
of a 96 well microtiter plate and incubated overnight at 4°C. Bovine serum albumin  
10 (2% in TrisCa buffer) was added to each well and incubated at room temperature for at  
least 2 hours in order to reduce nonspecific binding. CD34+ cells (10<sup>6</sup> cells/ml),  
purchased from Poetic Technologies Inc. (Gaithersburg, MD) were incubated with  
calcein AN (Molecular Probes, Eugene OR) diluted in Dulbecco's PBS (DPBS) at a  
concentration of 2µg/ml for 20 minutes at 37°C. After washing the microtiter plate 5  
15 times with DPBS, 100 µl of cells were added to each well. The plate was incubated in a  
stationary position for 25 minutes at room temperature. After incubation, the plate was  
inverted in a wash chamber (GlycoTech Corp., Rockville MD), for exactly 6 minutes.  
The plate was then removed from the chamber, and fluorescence of each well was  
determined at excitation and emission wavelengths of 485 and 530, respectively  
20 (Cytofluor 2350, PerSeptive Biosystems).

The results are presented in Figures 2 and 3. CD34+ cells bound to the  
attached antibody NUH2 and to the lectins *Maackia amurensis* lectin, Tomato lectin and  
sialoadhesin, but not to negative control proteins (BSA and IgM). These results indicate  
that the antibody and lectin binding partners can immobilize hematopoietic stem cells.

25

Example 3IDENTIFICATION OF HEMATOPOIETIC STEM CELLS USING AN  
ANTI-CARBOHYDRATE ANTIBODY

This Example illustrates the use of a representative antibody binding  
30 partner to identify hematopoietic stem cells by fluorescence activated cell sorting  
(FACS).

The expression of markers on the surface of hematopoietic stem cells was analyzed by a fluorescence activated cell sorter using red and green fluorescence. Human cord blood was washed twice with Dulbecco's PBS containing 0.1% sodium azide, pH 7.4. The cells were then divided into separate tubes. Monoclonal antibody  
5 NUH2 and a control IgM were each added to a different tube at 10ug/ml. After incubation at 4°C for 1 hour, the cells were washed and incubated with FITC-labeled goat anti-mouse IgM and FITC-labeled streptavidin (1µg/ml) respectively (KPL Labs, Gaithersburg, MD). After incubation at 4°C for 1 hour, the cells were washed twice with DPBS + azide and the commercial PE-labeled (red fluorescence) anti-CD34  
10 antibody, HPCA2-PE (Becton Dickinson, San Jose, CA) was added to each tube. After incubation for 30 minutes at 4°C, the cells were washed and the red blood cells were lysed by the reagent ACK Lysing Buffer (Biofluids, Rockville, MD). Cells in each tube were then fixed by the addition of Formalin.

The results are presented in Figures 4 and 5, which display data obtained  
15 from light scattered by lymphocytes in human blood from umbilical cords. The data are presented as contour plots which represent the bivariate distribution of green and red fluorescence. Contour lines known as isopleths are drawn to express a cell count at a particular set number. The X-axis is a logarithmic scale of green fluorescence labeled on cell surfaces, whereas the Y-axis is a logarithmic scale of red fluorescence labeled on  
20 cell surfaces. Quadrant 2 contains data that statistically demonstrate the presence of cells that express both surface markers, each detected by either the red or green fluorescent labeled antibody.

As shown in Figure 4, green fluorescent antibody NUH2 binds to almost all CD34+ cells that are identified by the red fluorescent antibody HPCA2-PE. Control  
25 IgM antibodies did not label these cells (Figure 5).

#### Example 4

#### IDENTIFICATION OF PLANT LECTIN BINDING PARTNERS

This Example illustrates the evaluation of representative lectin binding  
30 partners.

Plant lectins that bind CD34+ cells were also identified by two color FACS analysis. Using the method described above the cells in human cord blood were labeled with green fluorescence-labeled plant lectins followed by the red fluorescence-labeled anti-CD34 antibody, HPCA2-PE. Results of the scatter diagrams are summarized in Table 1 below. The binding of lectins to CD34+ cells is consistent with the expression of sialylated lactosamine structures on CD-34+ cells.

Table 1

Reactivity of Plant Lectins with Hematopoietic Stem Cells

Lectin	Specificity	Reactivity
Maackia amurensis	NeuAc $\alpha$ 2-3Gal	+
Sambucus nigra	NeuAc $\alpha$ 2-6Gal	+
Triticum vulgare (WGA)	NeuAc, GlcNAc $\beta$ 1-4GlcNAc	+
Lycopersicon esculentum (tomato)	(Gal $\beta$ 1-4GlcNAc) <sub>n</sub> , polylactosamine	+
Erytheina cristagalli (ECA)	Gal $\beta$ 1-4GlcNAc	+
Ulex europaeus (UEA)	$\alpha$ -Fucose	-
Lotus tetragonolobus	$\alpha$ -Fucose	-
Arachis hypogea (PNA)	Gal $\beta$ 1-3GalNAc	-

The positive results indicate that these lectin binding partners bind sialylated lactosamine structures.

Example 5

DETECTION OF CELL SURFACE ANTIGENS ON CULTURED CELLS BY IMMUNOFLUORESCENCE STAINING AND FLUORESCENCE

15

ACTIVATED CELL SORTING (FACS)

This Example illustrates the use of antibodies to detect cell surface structures on a variety of cultured cell lines by immunofluorescent staining followed by fluorescence activated cell sortings (FACS).

The expression of markers on the surface of cultured cell lines was analyzed by indirect immunofluorescent staining of the cell lines by specific monoclonal antibodies followed by fluorescence activated cell sorting. The following cell lines were obtained from the ATCC (American Type Culture Collection, Manassas, VA). The ATCC designation follows the cell type abbreviation: KG1a, CCL246.1; 5 HL60, CCL240; NCI-H446, HTB171; NCI-H82, HTB175; MCF7, HTB22 and Molt4, CRL1582. Adherent cell lines were removed from tissue culture surfaces and disaggregated by pipetting in 1X Versene (PBS-EDTA solution, Gibco, Life Technologies, Grand Island, NY). Cells were washed in phosphate-buffered saline 10 (PBS) and resuspended in Dulbecco's PBS (DPBS) containing 0.1% sodium azide and 1% bovine serum albumin (BSA). The cells were then divided into 3 separate tubes. Monoclonal antibodies NUH2, anti-Le<sup>x</sup>, and a control mouse IgM were each added to separate tubes at final concentration of 10µg/ml. After incubation at 4°C for 1 hour, the cells in each tube were washed with DPBS + azide and incubated with FITC-labeled 15 goat anti-mouse IgM (KPL Labs, Gaithersburg, MD) at a final concentration of 1 µg/ml. After incubation for 45 minutes at 4°C, the cells were washed twice with DPBS + azide and resuspended in DPBS + azide and 1% BSA. Labeled cells were maintained at 4°C and analyzed immediately by fluorescence activated cell sorting gated for fluorescein.

Cells were directly immunostained for antigens, CD34 and Thy-1 using 20 fluorescence-labeled antibodies anti-HPCA2-FITC and anti-Thy-1-PF, respectively. For these two antibodies, cells prepared as described above were incubated at 4°C for 1 hour in DPBS containing 0.1% sodium azide, 1% BSA and 10 µg/ml of staining antibody for either CD34 (anti-HPCA2-FITC, Becton-Dickenson, San Jose, CA) or Thy-1 (anti-CDw90-PE, Serotec, U.K.). After incubation, the cells were washed twice 25 with DPBS + azide and resuspended in DPBS + azide and 1% BSA. Labeled cells were maintained at 4°C and analyzed immediately by fluorescence activated cell sorting gated for either fluorescein or phycoerythrin, respectively.

The results are presented in Table 2 below. Each of the antibodies detects large differences in antigen presentation among the different cell lines. Both 30 Thy1 and the anti-carbohydrate antibody, anti-Le<sup>x</sup> [Galβ1-4(Fucα1-2)GlcNAcβ1-3Galβ1-R] bind to cell lines differently than the anti-carbohydrate antibody NUH2

(NeuAc $\alpha$ 2-3Gal $\beta$ 1-R]. In particular, KG1a cells, which express high levels of CD34 on their surfaces, are clearly devoid of the carbohydrate marker for hematopoietic stem cells, NeuAc $\alpha$ 2-3Gal $\beta$ 1-4-R, detected by antibody NUH2. Thus, the hematopoietic stem cell carbohydrate marker, NeuAc $\alpha$ 2-3Gal $\beta$ 1-4-R is clearly different than the classic antigen for hematopoietic stem cells, CD34.

Table 2

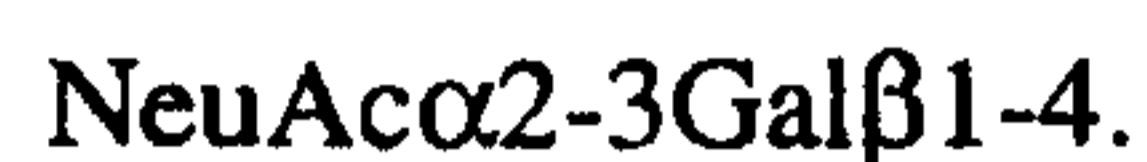
FACS Analysis of Cell Lines with Various Antibodies (% positive)

Antibody	KG1a	HL-60	NCI-H446	NCI-H82	MCF7	Molt4
CD34	99	<5	<5	<5	<5	23
anti-Lex	17	65	75	<5	60	31
NUH2	<5	<5	25	<5	<5	<5
Thy-1	<5	<5	39	35	<5	<5

10 From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

## CLAIMS

1. A method for immobilizing or purifying hematopoietic stem cells or targeting an agent to such cells, comprising the step of contacting a biological sample containing hematopoietic stem cells with a binding partner, wherein said binding partner binds to a sialylated carbohydrate chain comprising the structure:



2. A method according to claim 1, further comprising the step of separating the stem cell-binding partner complex formed in the first step from unbound biological sample.

3. A method according to claim 2, further comprising the step of separating said hematopoietic stem cell from said binding partner.

4. A method according to any one of claims 1, 2 or 3, wherein said binding partner is attached to a solid support after formation of a binding partner-stem cell complex.

5. A method according to any one of claims 1, 2 or 3, wherein said binding partner is attached to a solid support prior to formation of a binding partner-stem cell complex.

6. A method according to claim 4 or claim 5, wherein said solid support is a tissue culture dish.

7. A method according to claim 4 or claim 5, wherein said solid support is a chromatographic support.

8. A method according to claim 4 or claim 5, wherein said solid support is a hollow fiber bioreactor.

9. A method according to claim 4 or claim 5, wherein said solid support is a bag.
10. A method according to claim 1 wherein said binding partner is associated with an agent.
11. A method according to claim 10, wherein said agent comprises a toxic compound.
12. A method according to claim 10, wherein said agent comprises a therapeutic compound.
13. A method according to claim 10, wherein said agent comprises a detectable marker.
14. A method according to claim 10, wherein said agent comprises a polynucleotide.
15. A method according to any one of claims 1-14, wherein said sialylated carbohydrate chain comprises the structure:
- $$\text{NeuAc}\alpha 2\text{-3}(\text{Gal}\beta 1\text{-4GlcNAc}\beta 1\text{-3})_n$$
- wherein n is an integer ranging from 1 to 100.
16. A method according to claim 15, wherein n ranges from 1 to 50.
17. A method according to any one of claims 1-16, wherein said biological sample is selected from the group consisting of blood, bone marrow, buffy coat cells, cord blood and cells grown or expanded *in vitro*.

18. A method according to any one of claims 1-17, wherein said binding partner comprises an antibody or an antigen-binding fragment thereof.

19. A method according to claim 18, wherein said antibody or fragment thereof is a monoclonal antibody or an antigen-binding fragment thereof.

20. A method according to any one of claims 1-17, wherein said binding partner comprises a lectin.

21. A method according to claim 20, wherein said lectin is a plant lectin or a derivative thereof.

22. A method according to claim 21, wherein said plant lectin is selected from the group consisting of Tomato lectin, *Maackia amurensis* lectin, *Sambucus nigra* lectin, *Triticum vulgare* lectin, *Erythrina cristagalli* lectin and combinations thereof.

23. A method according to claim 20, wherein said lectin is a mammalian lectin or a derivative thereof.

24. A method according to claim 23, wherein said mammalian lectin is selected from the group consisting of sialoadhesins, galectins and combinations thereof.

25. A method according to claim 20, wherein said lectin is a bacterial lectin or a derivative thereof.

26. A method according to claim 25, wherein said bacterial lectin is a *Helicobacter pylori* carbohydrate receptor.

27. A pharmaceutical composition, comprising:

(a) an agent associated with a binding partner for stem cells, wherein said binding partner binds to a sialylated carbohydrate chain comprising the structure:

NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and

(b) a pharmaceutically acceptable carrier.

28. A pharmaceutical composition according to claim 27, wherein said agent comprises a therapeutic compound.

29. A pharmaceutical composition according to claim 27, wherein said agent comprises a detectable marker.

30. A pharmaceutical composition according to claim 27, wherein said agent comprises a polynucleotide.

31. A kit for use in immobilizing hematopoietic stem cells, comprising:

(a) a binding partner for stem cells, wherein said binding partner binds to a sialylated carbohydrate chain comprising the structure:

NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and

(b) a wash solution,

wherein said binding partner and said wash solution are provided in separate compartments or containers.

32. A kit according to claim 31, wherein said binding partner is attached to a tissue culture dish.

33. A kit for use in separation or purification of stem cells, comprising:

(a) a binding partner for stem cells, wherein said binding partner binds to a sialylated carbohydrate chain comprising the structure:

NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and

(b) a solid support.

34. A kit for use in labeling or sorting hematopoietic stem cells, comprising

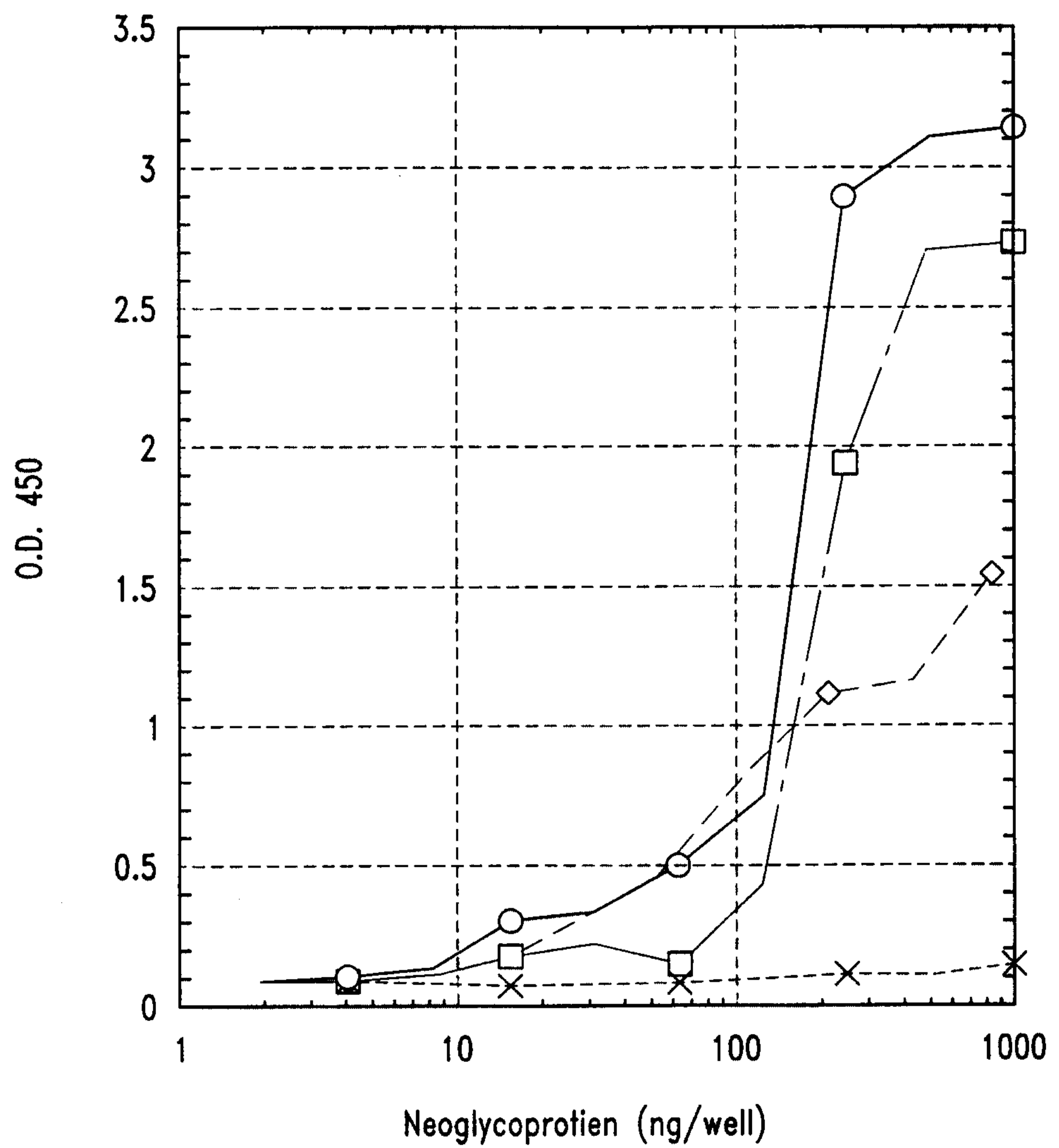
(a) a binding partner for stem cells associated with a detectable marker, wherein said binding partner binds to a sialylated carbohydrate chain comprising the structure:

NeuAc $\alpha$ 2-3Gal $\beta$ 1-4; and

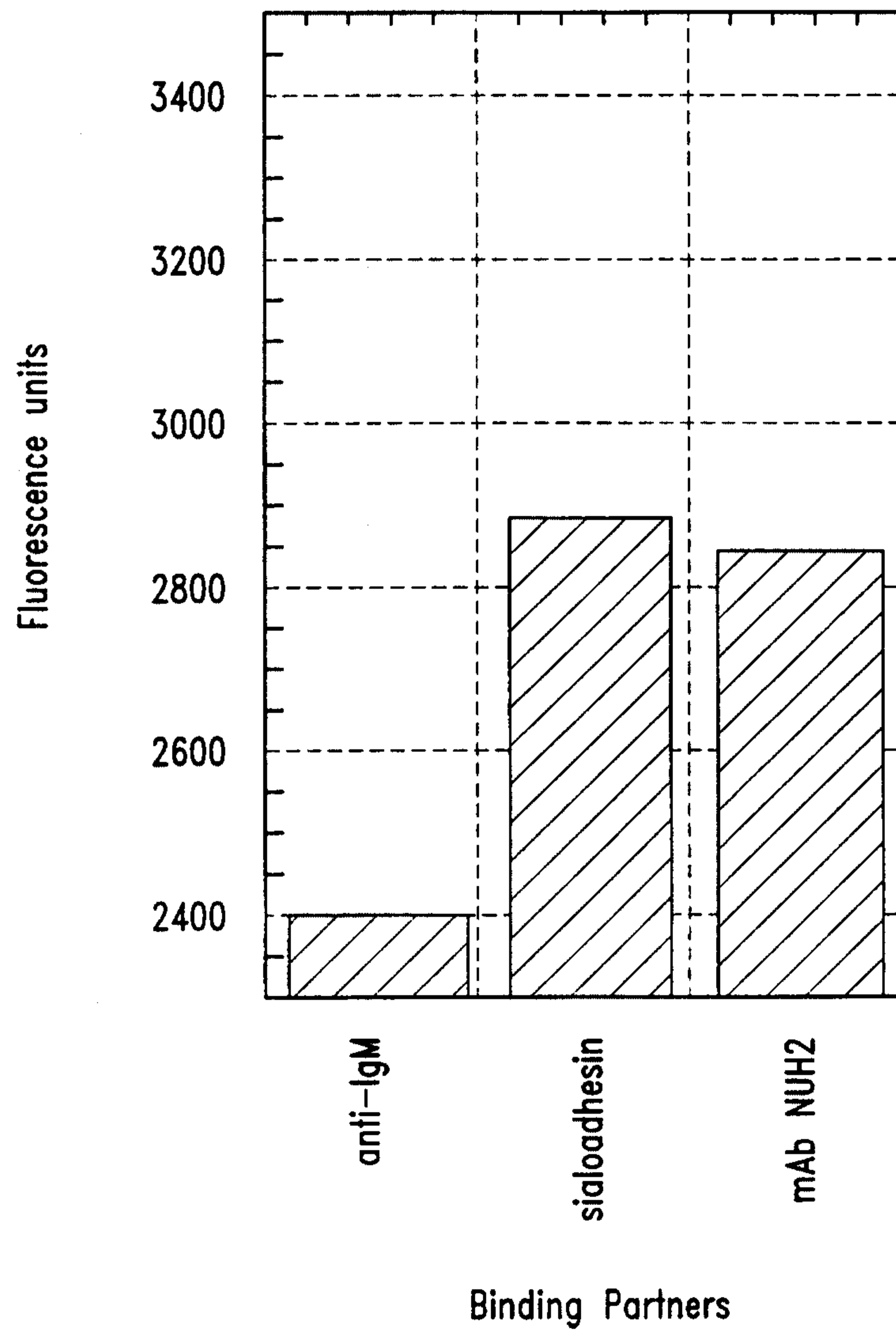
(b) a detection reagent,

wherein said binding partner and said detection reagent are provided in separate compartments or containers.

1/5

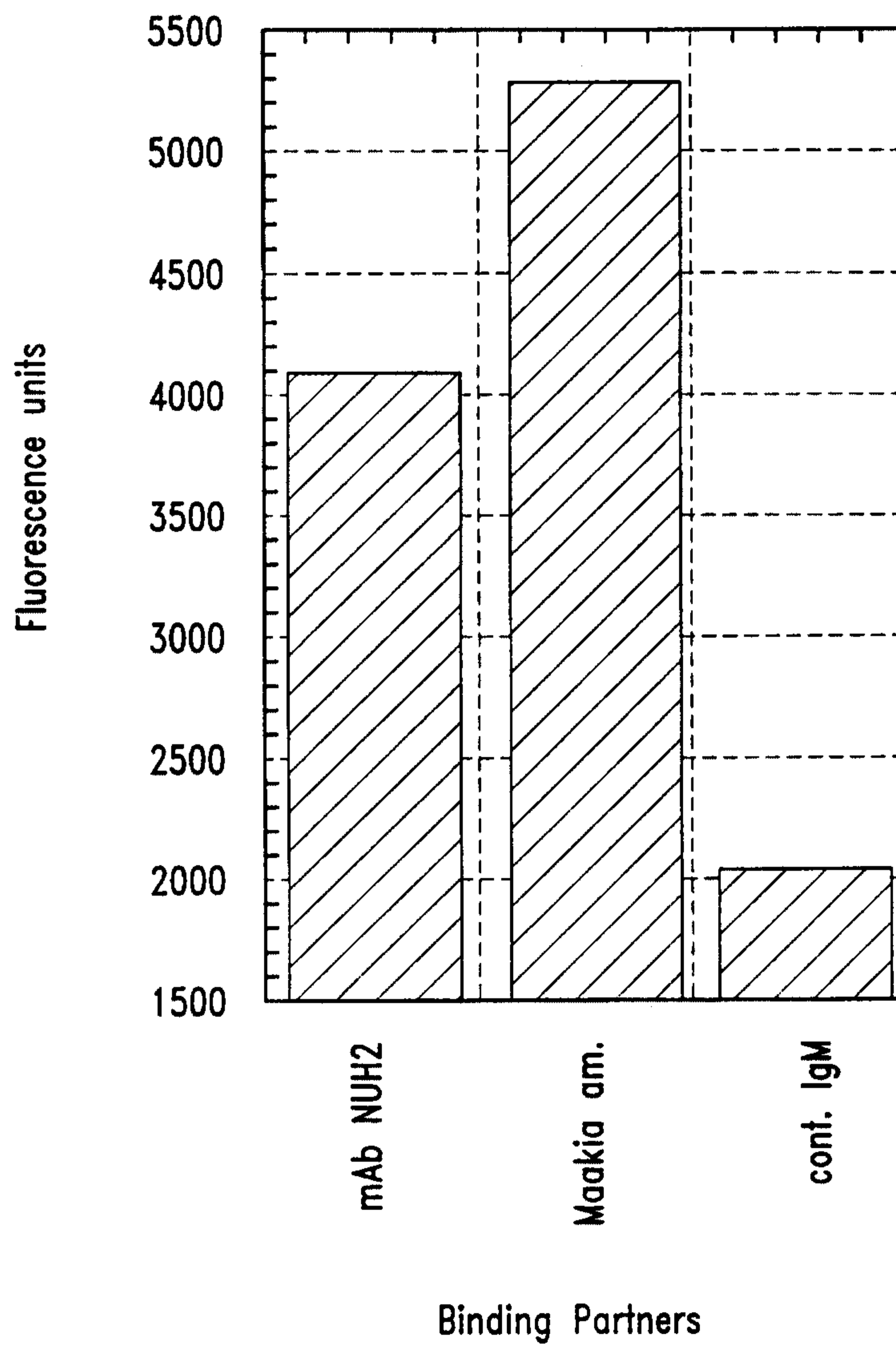
Binding of antibody NUH<sub>2</sub> to Neoglycoproteins*Fig. 1*

2/5



*Fig. 2*

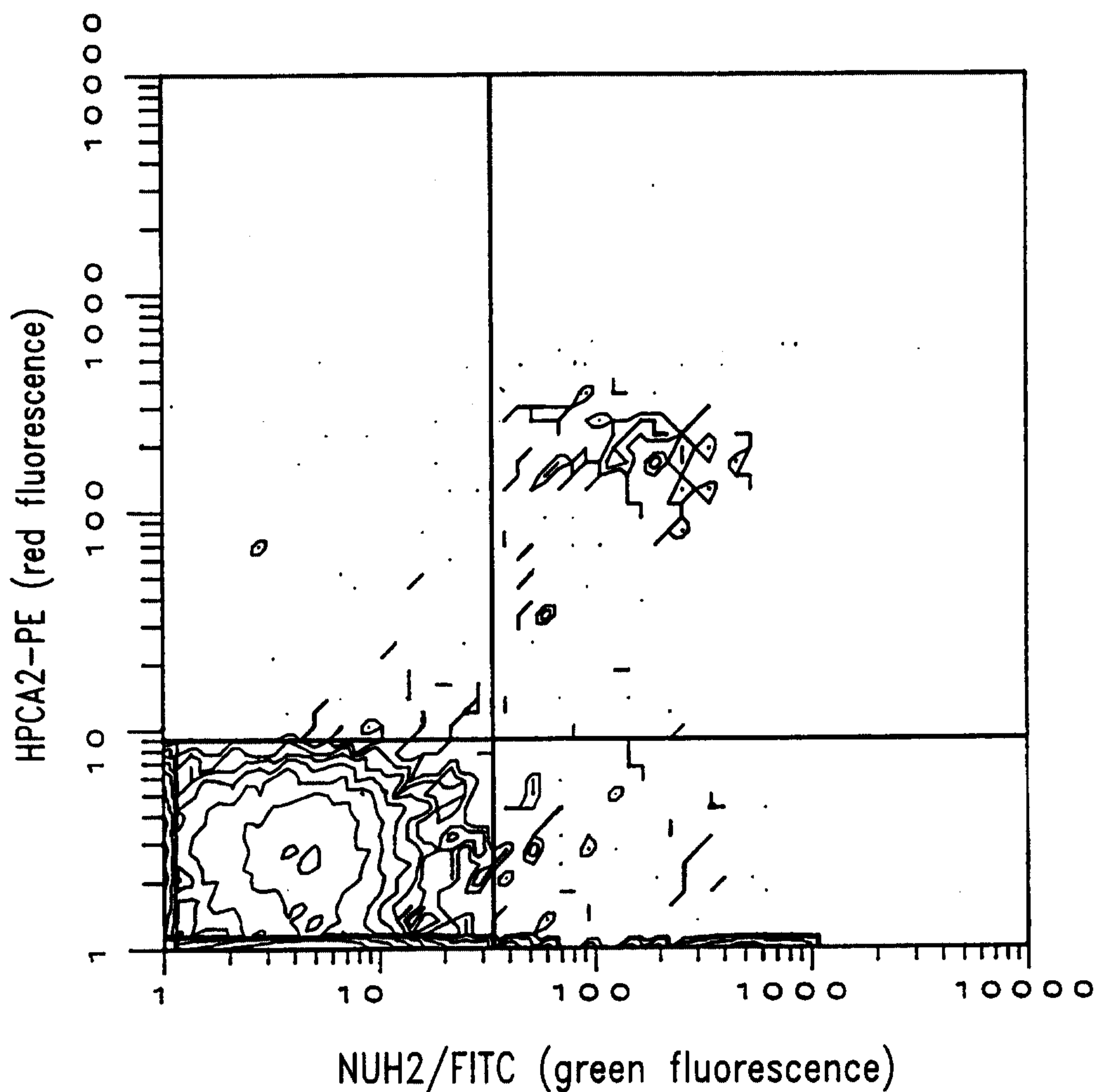
3/5



*Fig. 3*

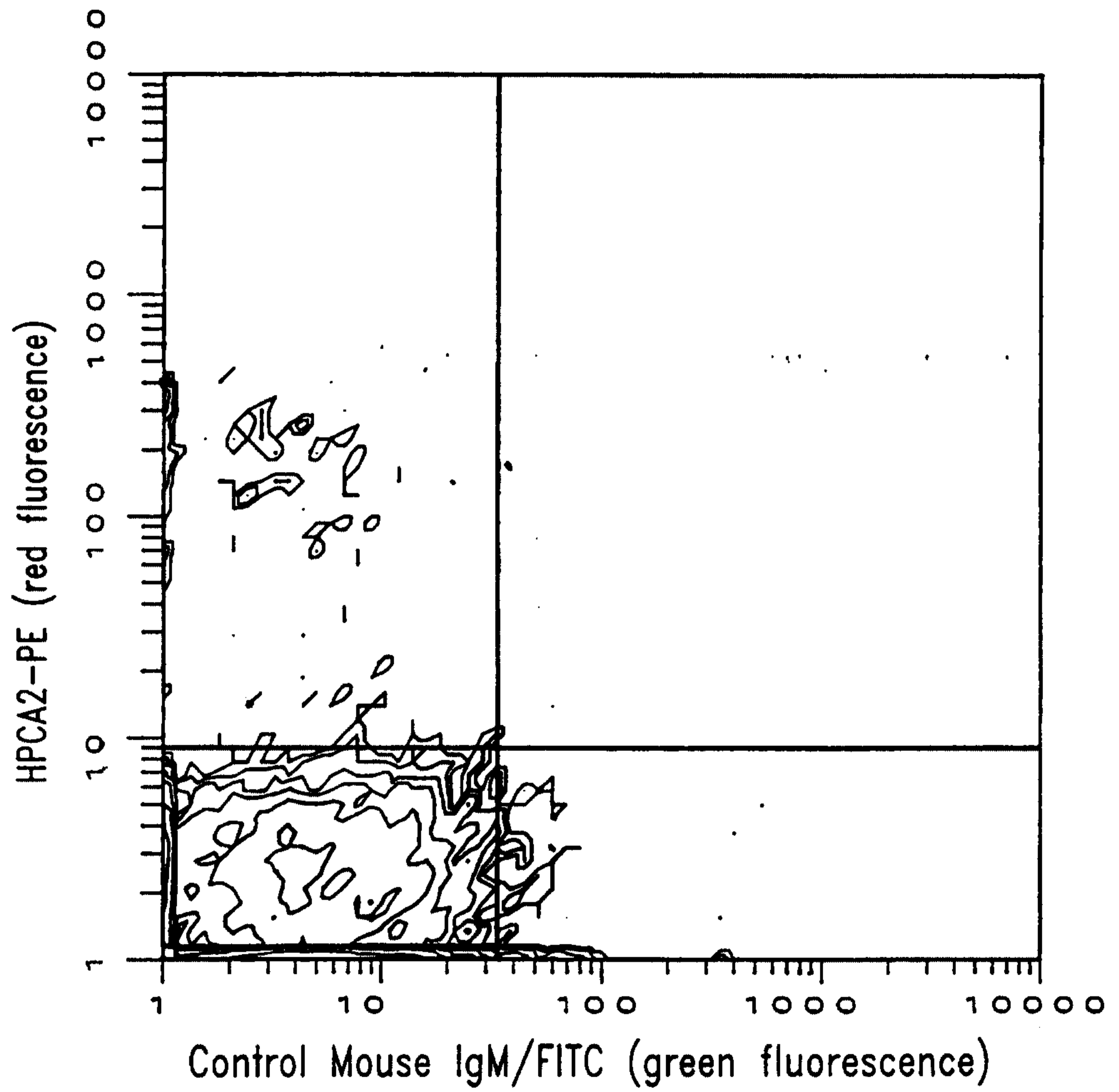
4/5

Monoclonal Antibody NUH2 (anti-sialyl type 2) Identifies Hematopoietic Stem Cells as Determined by 2 Color FACS Analysis Using Antibody HPCA2 (anti-CD34)

*Fig. 4*

5/5

Control Mouse IgM Does Not Label Hematopoietic Stem Cells  
as Determined by 2 color FACS Analysis  
Using Antibody HPCA2 (anti-CD34)

*Fig. 5*