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TECHNICAL DATA SHEET 587

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BioMag[®] SelectaPure[™] Anti-CD34 Antibody

Description

CD34 antigen is expressed on hematopoietic progenitor cells of all lineages and on the most primitive pluripotent stem cells. CD34 expression is highest on the earliest stem cells and is gradually lost as the progenitor cells become committed and differentiate. CD34 antigen is also expressed on capillary endothelial cells and on bone marrow stromal cells.

CD34 antigen is a monomeric transmembrane phosphoglycoprotein of approximately 110 kDa. The extracellular portion contains two distinct domains, the membrane proximal domain, about 110 amino acids, and the NH₂-terminal domain, about 140 amino acids and is heavily glycosylated with N-linked glycans and sialylated O-linked carbohydrates. Variations in glycosylation occur during normal hematopoiesis depending on commitment to lineage and the level of maturation. The proximal domain probably exists in a globular conformation and the NH₂ terminal domain likely exhibits an extended rod-like structure similar to the mucin-like glycoproteins.

BioMag Anti-CD34 particles are designed for the positive isolation of myeloid progenitor cells.

Particle Concentration

The concentration of BioMag is approximately 4 to 5 mg/ml. There are approximately 1×10^8 BioMag particles per mg.

Cell Separation Recommendations

Depending upon antigen availability and the size of the target cell population, cell sorting applications may require up to 50-60 magnetic particles per cell based on the target cell population. Magnetic particles and cells should be incubated at room temperature for 30 minutes to one hour in media containing 5-10% protein (to reduce non-specific binding) for successful separation. Gentle end over end or rocking during incubation is required for optimal results. (Note: Increasing the incubation time beyond one hour may be necessary to achieve the desired depletion.) Each researcher must optimize particle to cell ratio and incubation time for the application.

Some applications require the detachment of BioMag antibody particles from cells after separation. One approach would involve culturing cells after positive selection. Cultures can be maintained for about 48 hours during which magnetic particles fall away from cells due to cell surface changeover. The magnetic particles are then easily removed via a magnetic separation. Another approach is the use of a protease such as chymopapain to break the antigen-antibody bond and remove the particles magnetically. Depending upon the application, it may not be necessary to remove the cells from the BioMag particles. BioMag particles are only 1 μ m in size and have been successfully used in FACS equipment. They will not jam the machine and are distinguishable from cells. Alternatively, negative selection approaches can be very effective in producing specific cell populations.

Storage and Stability

The suspension is supplied in PBS/EDTA/1.0% BSA/0.1% sodium azide buffer at pH 7.5. Washing BioMag Anti-CD34 particles in sterile media to remove preservative prior to use is recommended. Using a magnetic separation unit for washing instead of centrifugation is strongly recommended. Do not freeze, dry or centrifuge BioMag. Freezing, drying and centrifuging BioMag can result in aggregation and loss of binding activity. BioMag Anti-CD34 particles are stable when stored at 4°C.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

Figure A-1

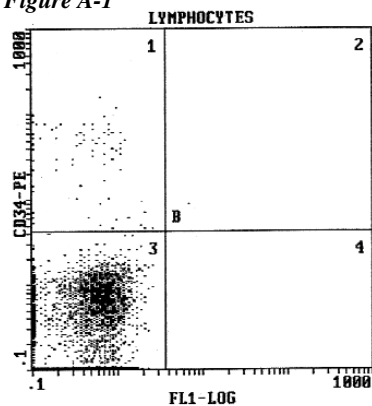
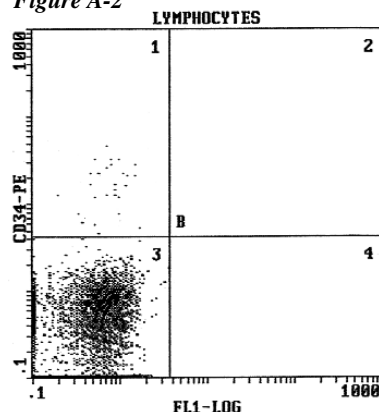


Figure A-2



General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.05 ml
# Particles	2.00 x 10 ⁷ per test
# Target Cells	1.00 x 10 ⁵ per test
Bead:Cell Ratio	200:1
% Depletion	73.2%

*These values should be used as a starting point in optimizing experimental protocols. Due to differences in the distribution of cell types in samples and other variables, the researcher is strongly encouraged to determine the optimal particle to cell ratios for their experiments.

Figure B-1

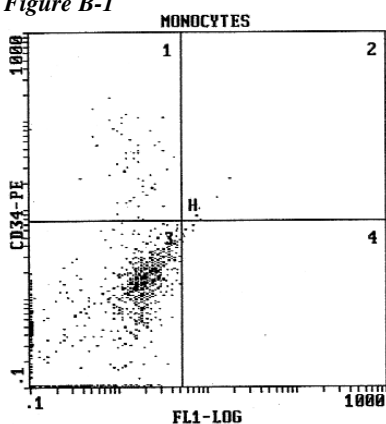
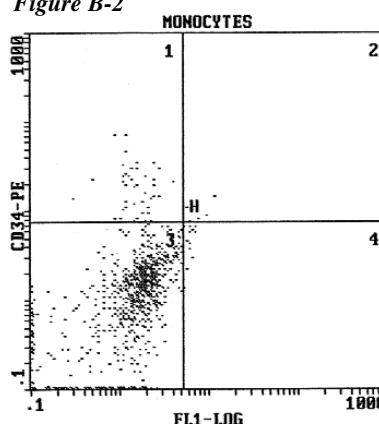


Figure B-2



General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.05 ml
# Particles	2.00 x 10 ⁷ per test
# Target Cells	5.00 x 10 ⁴ per test
Bead:Cell Ratio	400:1
% Depletion	50%

*These values should be used as a starting point in optimizing experimental protocols. Due to differences in the distribution of cell types in samples and other variables, the researcher is strongly encouraged to determine the optimal particle to cell ratios for their experiments.

Cell sorting results using BioMag Selectapure CD34 anti-human leukocyte particles for positive selection. Typically, bone marrow monoclonal preparations and particles are incubated for 30 minutes at room temperature and then magnetically separated. The supernatant is collected, incubated with the appropriate two-color antibody cocktail, and then analyzed by flow cytometry. Figures A-1 and B-1 depict the cell population prior to positive selection. Figures A-2 and B-2B depict the cell population after positive selection. The particle to cell ratios reported above are based on experiments where cells were exposed to the particles once.

Safety

BioMag Anti-CD34 particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

Ordering Information:

Cat. #	Description	Size
85034	BioMag Anti-CD34 Antibody	1 ml 5 ml

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
 In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
 In Germany FAX: (49) 6221-764620

Order online anytime at www.polysciences.com

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