Product Data Sheet 587

BioMag® anti-Human CD34

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

The 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 NH-2 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 level of maturation. The proximal domain probably exists in a globular conformation and the NH-2 terminal domain likely exhibits an extended rod-like structure similar to the mucin-like glycoproteins.

BioMag® anti-Human CD34 particles are designed for the positive isolation of myeloid progenitor cells.

BioMag® anti-Human CD34 is a suspension of magnetic particles approximately 1.5µm in size. The suspension is supplied in a phosphate buffered saline (pH 7.5) containing EDTA, 1.0% BSA, and 0.1% sodium azide.

CHARACTERISTICS

Mean Diameter:	~1.5µm
Particle Concentration:	4 mg/mL
Particle Count:	1 x 10^8 BioMag® particles per mg

PROCEDURE

Researchers are advised to optimize the use of particles in any application.

Depending on antigen availability and the size of the target cell population, cell sorting applications may require up to 50-60 magnetic particles per cell. Magnetic particles and cells should be incubated at room temperature for 30-60 minutes in media containing 5-10% protein (to reduce nonspecific binding) for successful separation. Gentle end-over-end mixing 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.)

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

Cell sorting results using BioMag® anti-Human CD34 leukocyte particles for positive selection. *Typically, bone marrow mononuclear 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 populations prior to positive selection. Figures A-2 and B-2 depict the cell populations after positive selection. The particle to cell ratios reported above are based on experiments where cells were exposed to the particles once.*

** The values under "General Recommendations" 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 A-1

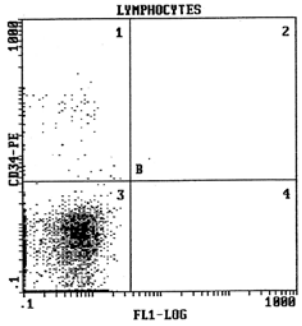
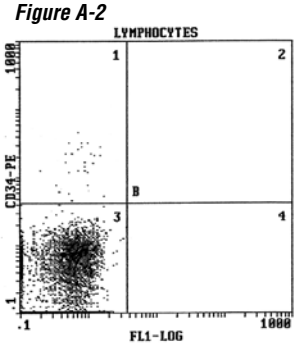


Figure A-2



General Recommendation*:

Concentration#	4.0 x 10^8 particles/mL
Volume Used	0.05mL
# Particles	2.00 x 10^7 per test
# Target Cells	1.00 x 10^5 per test
Particle:Target Cell Ratio	200:1
% Depletion	73.2%

Figure B-1

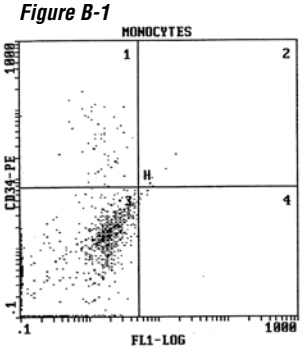
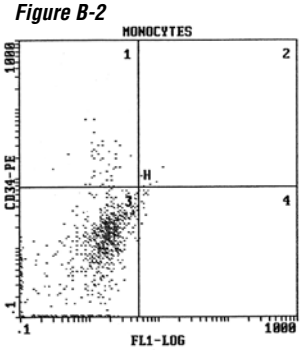


Figure B-2



General Recommendation*:

Concentration#	4.00 x 10^8 particles/mL
Volume Used	0.05mL
# Particles	2.00 x 10^7 per test
# Target Cells	5.00 x 10^4 per test
Particle:Target Cell Ratio	400:1
% Depletion	50.00%

upon the application, it may not be necessary to remove the cells from the BioMag® particles. BioMag® particles are only 1-2µm 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

Store at 2-8˚C. Freezing, drying, or centrifuging BioMag® may result in irreversible aggregation and loss of binding activity. Washing BioMag® anti-Human CD34 particles in sterile media to remove preservative prior to use is recommended. Using a magnetic separation unit for washing instead of centrifugation is also strongly recommended.

SAFETY

This 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.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
BM587	BioMag® anti-Human CD34	5mL

Order online anytime at **www.bangslabs.com.**