Product Data Sheet 588

BioMag® anti-Human CD45

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

CD45, a major component of the lymphocyte membrane, is present on the surface of all human leukocytes, lymphocytes, eosinophils, monocytes, basophils, and neutrophils. In addition, CD45 antibodies react with the leukocyte progenitors found in bone marrow. CD45 is lost during maturation of erythroid cells in the bone marrow and is absent from erythrocytes and platelets.

The group of five CD45 isoforms are single chain integral membrane proteins, ranging from 180 to 220 kDa. The different isoforms are generated by alternative splicing of three exons of the genomic sequence. The Leukocyte Common Antigen consists of an extracellular proximal membrane sequence common to all CD45 isoforms. All the monoclonal antibodies of the CD45 cluster react with this part of the antigen and thus are able to recognize all of the CD45 isoforms. The different isoforms have extra cytoplasmic sequences ranging from 391 to 552 amino acids, which have numerous N-linked carbohydrate attachment sites. The cytoplasmic portion of CD45 contains two phospho-tyrosine-phosphatase domains.

BioMag® anti-Human CD45 particles recognize the Leukocyte Common Antigen and are used for bone marrow and peripheral blood depletion of malignant and normal leukocytes, including B cells and granulocytes. BioMag® anti-Human CD45 particles may also be useful in studies involving T cell activation and inhibition.

BioMag® anti-Human CD45 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

Cell sorting results using BioMag® anti-Human CD45 leukocyte particles for positive selection. *Typically, whole blood or purified leukocytes 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. Figure A-1 depicts the neutrophil population prior to positive selection. Figure A-2 shows the cell population after positive selection with BioMag anti-Human CD45 particles. Figure A-3 shows the cell population after positive selection with CD45 and CD16 combined in equal ratios. Figures B-1 through B-3 depict the results for lymphocytes and Figures C-1 through C-3 depict the results for monocytes. The particle to cell ratios reported above are based on experiments where cells were exposed to the particles once.*

Figure A-1

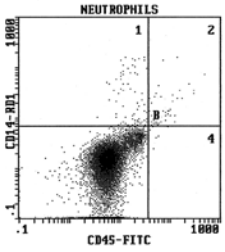


Figure A-2

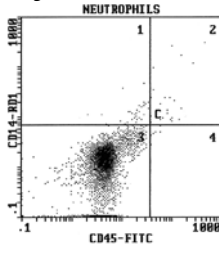


Figure A-3

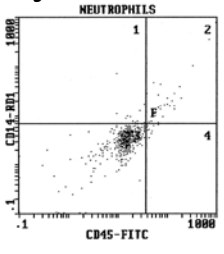


Figure B-1

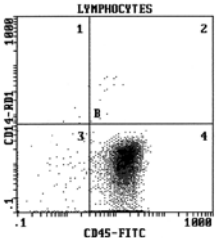


Figure B-2

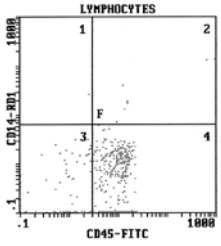


Figure B-3

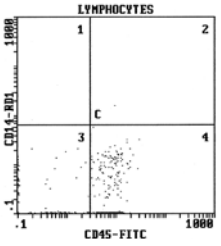


Figure C-1

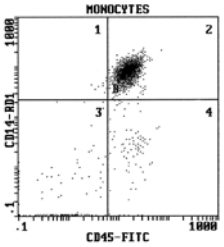


Figure C-2

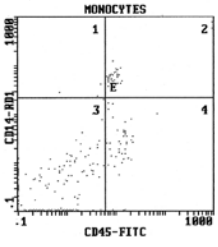
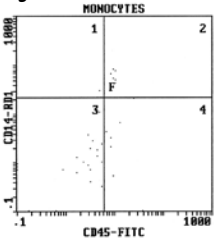


Figure C-3



General Recommendation – BioMag® CD45 for Lymphocytes*:

Concentration#	4.0 x 10^8 particles/mL
Volume Used	0.200mL
# Particles	8.00 x 10^7 per test
# Target Cells	1.72 x 10^6 per test
Particle:Target Cell Ratio	46.5
% Depletion	97.36%

General Recommendation – BioMag® CD45 and CD16 for Lymphocytes*:

Concentration#	4.0 x 10^8 particles/mL
Volume Used	0.100mL (50mL each)
# Particles	4.00 x 10^7 per test
# Target Cells	1.72 x 10^6 per test
Particle:Target Cell Ratio	23.3
% Depletion	96.73%

** 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.*

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 1-2µm 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 CD45 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
BM588	BioMag® anti-Human CD45	5mL

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