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TECHNICAL DATA SHEET 585

BioMag[®] SelectaPure[™] Anti-CD16 Antibody

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Description

The CD16 antigen is analogous to the Fc gamma RIII receptor expressed on Natural Killer cells, polymorphonuclear neutrophils, and macrophages. CD16 acts as a major activating receptor for NK cells and plays a role in mediating antibody-dependent cytotoxicity.

CD16 exists in two different isoforms, FcgammaRIIIA, a transmembrane form (50-65 kDa), and FcgammaRIIIB, a Glycosyl-Phosphatidylinositol (GPI) anchored form (48 kDa). The FcgammaRIIIA isoform is expressed on NK cells, monocytes and macrophages. FcgammaRIIIB is expressed mainly on neutrophils, and can be found as two allelic variants, Neutrophil Antigen 1 (NA1) and Neutrophil Antigen 2 (NA2). The two isoforms exhibit different glycosylation patterns. The FcgammaRIIIA molecule may be non-covalently associated with the gamma chain of FcepsilonRI, or the zeta chain of CD3 on NK lymphocytes but not on macrophages.

BioMag Anti-CD16 particles may be used in the study of antibody dependent complement cytotoxicity (ADCC) and to monitor NK cell levels in peripheral blood and tissue.

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 1µm 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-CD16 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-CD16 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

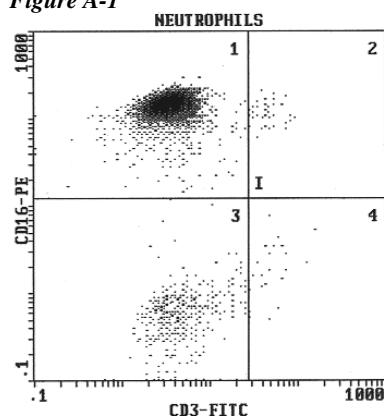
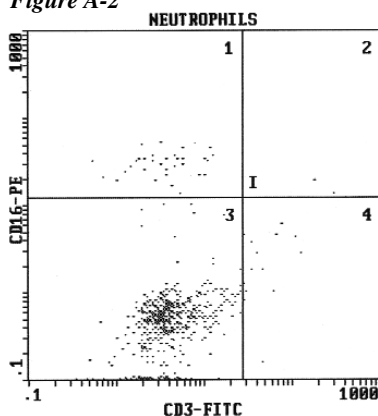


Figure A-2



General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.010 ml
# Particles	4.00 x 10 ⁶ per test
# Target Cells	2.76 x 10 ⁶ per test
Bead:Cell Ratio	1.5
% Depletion	99.30%

*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

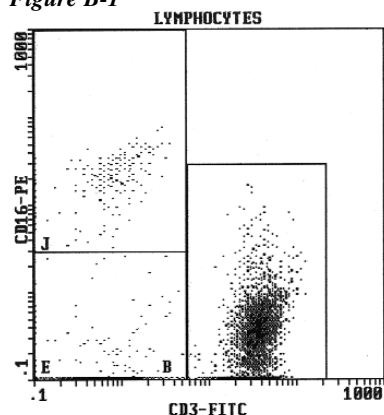
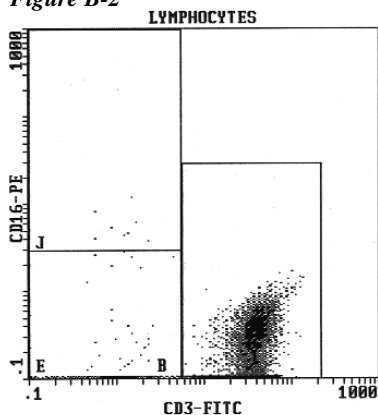


Figure B-2



General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.010 ml
# Particles	4.00 x 10 ⁶ per test
# Target Cells	1.12 x 10 ⁵ per test
Bead:Cell Ratio	35.7
% Depletion	97.30%

*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 CD16 anti-human 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 depicts the cell population prior to positive selection. Figure B depicts 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-CD16 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
85016	BioMag Anti-CD16 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

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