

BioMag[®] SelectaPure[™] Anti-CD8 Antibody

Description

The CD8 molecule is found on a T cell subset of human peripheral blood lymphocytes, the suppressor/cytotoxic T lymphocytes, and is widely used as a marker of this cell type. Certain NK cells may also express the CD8 antigen but with low to medium density of expression. CD8 is also present on most thymocytes where it is frequently co-expressed with CD4, and on a subpopulation of bone marrow cells. Many autoimmune diseases have been associated with a decrease in CD8 positive suppressor T lymphocytes.

The CD8 antigen is a disulfide-linked dimer, existing either as a CD8alpha homodimer or a CD8alphabeta heterodimer. The molecular weights of the alpha and beta subunits range from 32-34 kDa. CD8beta is required for surface expression of CD8alpha. The CD8 antibody clone B9.11 binds to the alpha3 domain of the MHC Class I molecules and acts with the T Cell Receptor as a co-receptor for MHC class I restricted antigen recognition.

CD8 BioMag Anti-CD8 particles can be useful in the detection of diseases such as multiple sclerosis, systemic lupus erythematosus, severe atopic eczema and others. BioMag Anti-CD8 is also useful in the recognition of different major histocompatibility complex regions.

Figure A

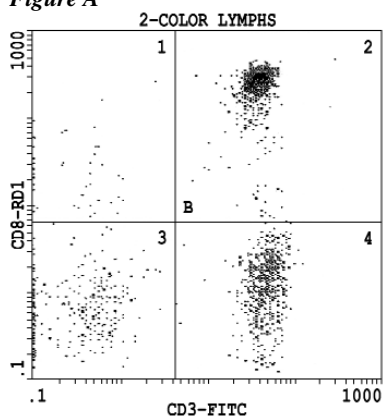
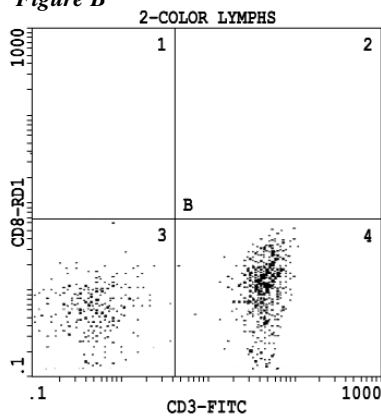


Figure B



General Recommendation*:

Conc. #	1.40 x 10 ⁸ bead/ml
Vol. Used	0.010 ml
# Particles	1.40 x 10 ⁶ per test
# Target Cells	2.73 x 10 ⁵ per test
Bead:Cell Ratio	5.1
% Depletion	98.24%

*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 CD8 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.

Particle Concentration

The concentration of BioMag is 1.5 mg/ml. There are approximately 1 x 10⁸ 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-CD8 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-CD8 particles are stable when stored at 4°C.

Safety

BioMag Anti-CD8 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
85008	BioMag Anti-CD8 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

This product is for research use only and is not intended for use in humans or for in vitro diagnostic use.

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.