

9025 Technology Dr. • Fishers, IN 46038-2886
 800.387.0672 • 317.570.7020 • Fax 317.570.7034
 info@bangslabs.com • www.bangslabs.com



B E A D S • A B O V E T H E R E S T [™]

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 CD8 alpha homodimer or a CD8 alphabeta heterodimer. The molecular weights of the alpha and beta subunits range from 32-34 kDa. CD8 beta is required for surface expression of CD8 alpha. 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.

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

BioMag[®] anti-Human CD8 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: ~1.5 mg/mL
 Particle Count: 1 x 10⁸ BioMag[®] particles per mg

PROCEDURE

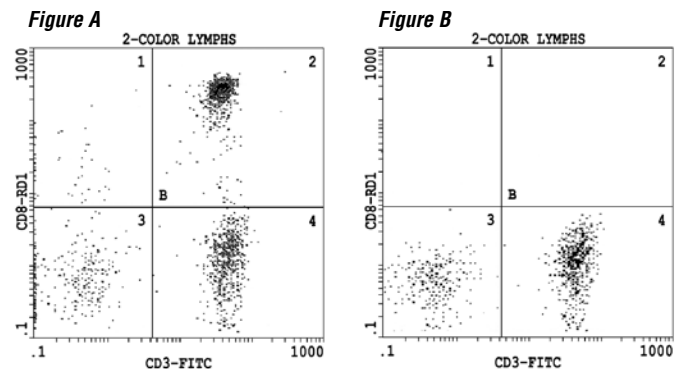
Researchers are advised to optimize the use of particles in any application.

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 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.

Cell sorting results using BioMag[®] anti-Human CD8 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.



General Recommendation*:

Concentration#	1.40 x 10 ⁸ particles/mL
Volume Used	0.01mL
# Particles	1.40 x 10 ⁶ per test
# Target Cells	2.73 x 10 ⁵ per test
Particle:Target 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.

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 CD8 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
BM583	BioMag® anti-Human CD8	5mL

Order online anytime at www.bangslabs.com.