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B E A D S • A B O V E T H E R E S T™

Description

The CD3 antigen is expressed by thymocytes, mature T cells, and early thymocytes. Because of this, CD3 has been utilized as an early marker of T cell differentiation. The CD3 antigen is composed of 5 polypeptide chains: gamma, delta, epsilon, zeta, and eta. The zeta chain exists as a homodimer or a heterodimer with the eta chain. In T cells, the CD3 antigen is part of a larger complex that includes the T cell receptor.

Antibodies to CD3 are useful in detecting normal and neoplastic T cells. The UCHT-1 antibody clone attached to a BioMag anti-Human CD3 particle reacts with the epsilon-chain of the CD3 complex. The UCHT-1 clone has a mitogenic effect on T lymphocytes and can be used to detect intracytoplasmic CD3 in flow cytometry after permeabilization. The UCHT-1 clone does not react with T cells lacking the TCR.

BioMag anti-Human CD3 particles are used for depletion of T cells from bone marrow and peripheral blood, the identification of T cells in tissues, peripheral blood T cell monitoring, and the study of mitogenic effects of anti-CD3 antibodies on T cells.

BioMag anti-Human CD3 is a suspension of magnetic particles approximately 1.5µm in size, which are coated with a mouse anti-Human T cell IgG1 UCHT-1 monoclonal antibody. The suspension is supplied in a phosphate buffered saline (pH 7.5) containing EDTA, 1.0% BSA, and 0.1% sodium azide.

Figure A

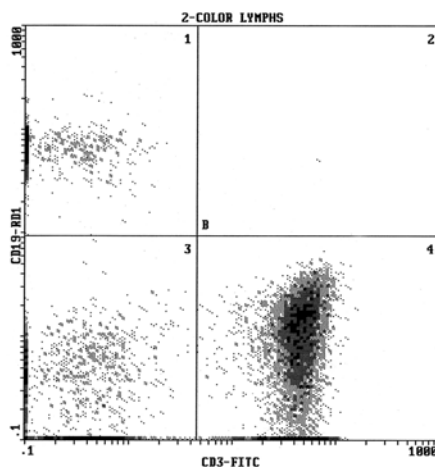
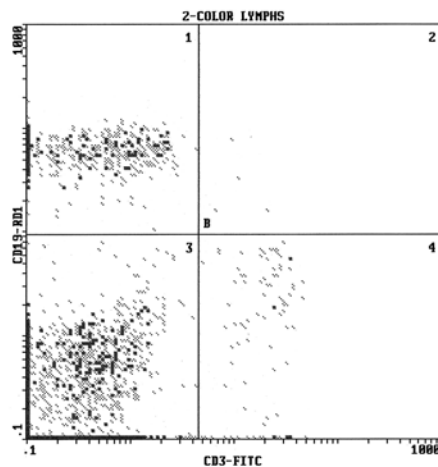


Figure B



General Recommendation*:

Concentration#	4.00 x 10 ⁸ particles
Volume Used	0.0125mL
# Particles	5.0 x 10 ⁶ per test
# Target Cells	1.05 x 10 ⁶ per test
Particle:Target Cell Ratio	4.8
% Depletion	98.82%

* 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® anti-Human CD3 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.



Characteristics

Mean Diameter: ~1.5µm
 Particle Concentration: ~4 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.

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 CD3 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.

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

Ordering Information

Catalog Code	Description	Size
BM580	BioMag® anti-Human CD3	5mL

Order online anytime at www.bangslabs.com.