

## BioMag<sup>®</sup> SelectaPure<sup>™</sup> Anti-CD3 Antibody (Pan T Cell)

### Product Description

The CD3 antigen is expressed by thymocytes and 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 bigger complex that includes the T Cell Receptor.

Antibodies to CD3 are useful in detecting normal and neoplastic T-cells. The UCHT1 antibody clone attached to BioMag Anti-Human CD3 particle reacts with the epsilon-chain of the CD3 complex. The UCHT1 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-CD3 is a suspension of magnetic particles approximately 1µm in size attached to a mouse anti-human T cell IgG1 UCHT-1 monoclonal antibody. BioMag Anti-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 study of the mitogenic effects of anti-CD3 antibodies on T cells.

Figure A

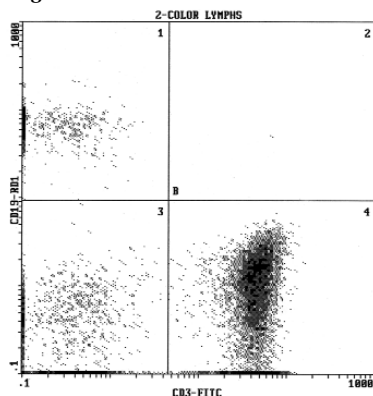
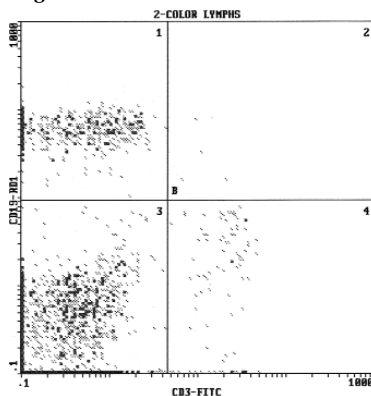


Figure B



### General Recommendation\*:

Conc. #	4.00 x 10 <sup>8</sup> bead/ml
Vol. Used	0.0125 ml
# Particles	5.0 x 10 <sup>6</sup> per test
# Target Cells	1.05 x 10 <sup>6</sup> per test
Bead: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 Selectapure CD3 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 approximately 4 to 5 mg/ml. There are approximately 1 x 10<sup>8</sup> 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-CD3 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-CD3 particles are stable when stored at 4°C.

### Safety

BioMag Anti-CD3 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
85003	BioMag Anti-CD3 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](http://www.polysciences.com)

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