

BioMag[®] SelectaPure[™] Anti-Mouse CD4 Antibody Particles

Description

The CD4/L3T4 antigen is a type I transmembrane glycoprotein expressed on most thymocytes and on the helper/inducer subpopulation of mature T lymphocytes. The CD4 antigen acts as a co-receptor that interacts with class II major histocompatibility complex molecules.

BioMag Anti-Mouse CD4 Antibody particles are designed for positive selection of CD4 plus mouse thymocytes and T Helper lymphocytes. The antibody reacts with the L3T4 antigen found on subpopulations of mouse T-cells, which includes thymocytes and T Helper cells. The antibody is not known to react with cytotoxic T-cells.

Figure A

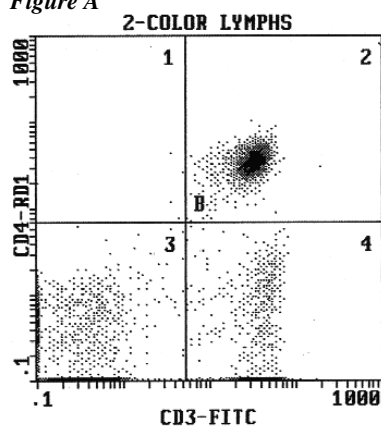
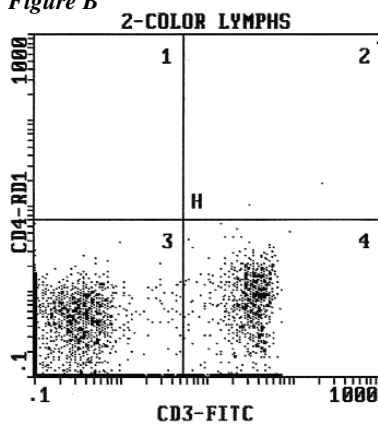


Figure B



General Recommendation*:

Conc. #	1.0 x 10 ⁸ particles/ml
Vol. Used	0.025 ml
# Particles	2.50 x 10 ⁶ /ml of blood
# Target Cells	Approximately 4.5 x 10 ⁵ /ml
Particles:Cell Ratio	6:1
% Depletion	98.6%

*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 anti-mouse CD4 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 Anti-Mouse CD4 is approximately 1.0 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-Mouse CD4 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 particles. Freezing, drying and centrifuging BioMag particles can result in aggregation and loss of binding activity. BioMag Anti-Mouse CD4 particles are stable when stored at 4°C.

Safety

BioMag Anti-Mouse CD4 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
85104	BioMag Anti-Mouse CD4 Antibody Particles	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

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