

BioMag[®] SelectaPure[™] Anti-Mouse CD45R Antibody Particles

Description

The B220 isoform of the CD45 antigen is a subset of the CD45 glycoprotein family that is expressed on all hematopoietic cells with the exception of mature erythrocytes.

BioMag Anti-Mouse CD45R Antibody Particles are designed for positive selection of hematopoietic cells with the exception of the mature erythrocytes. The antibody used reacts with cells expressing a CD45R (B220) phenotype, primarily cells of B-lineage, including pro B-cells through mature B-cells. The antibody can also react with a subset of activated T-cells and with non-MHC-restricted active lymphokine-activated killer cells.

Figure A

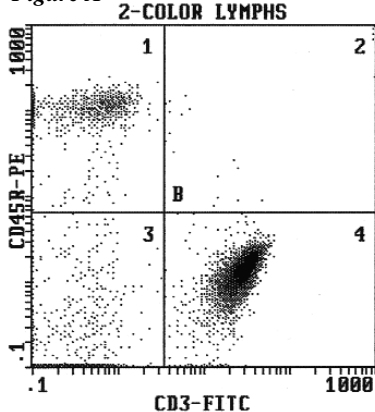
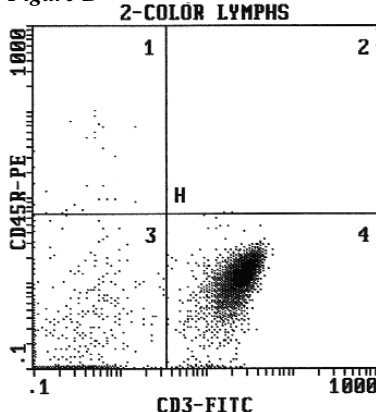


Figure B



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 2.5 x 10 ⁵ /ml
Particles:Cell Ratio	11:1
% Depletion	97.0%

*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 CD45R 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 CD45R 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 1 μm 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 CD45R 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 CD45R particles are stable until the expiration date indicated on the container when stored at 4°C.

Safety

BioMag Anti-Mouse CD45R 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
85145	BioMag Anti-Mouse CD45R	1 ml
	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

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