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TECHNICAL DATA SHEET 590

BioMag[®] SelectaPure[™] Anti-CD71 Antibody

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Description

The CD71 antigen is also known as the transferrin receptor. CD71 is expressed by activated T and B lymphocytes, macrophages and erythroblasts. CD71 expression is upregulated on all proliferating cell types and is absent from resting blood leukocytes. A type II membrane glycoprotein, it is a disulfide-bound dimer of two identical subunits of 95 kDa. The N-Terminal, intracytoplasmic domain of CD71 mediates rapid endocytosis and recycling.

BioMag Anti-CD71 particles recognize the 95 kDa transferrin receptor molecule present on proliferating cells including neoplastic cells, hematopoietic precursor cells and activated lymphocytes. Research applications include the identification of proliferating neoplastic cells of the hematopoietic and other organ systems.

Particle Concentration

The concentration of BioMag is approximately 4 to 5 mg/ml. There are approximately 1×10^8 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 μ m 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-CD71 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-CD71 particles are stable when stored at 4°C.

Safety

BioMag Anti-CD71 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.

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Figure A-1

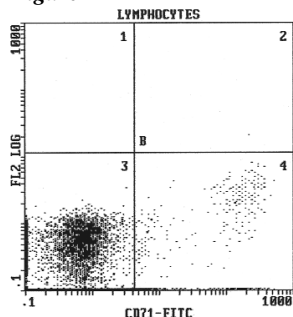


Figure B-1

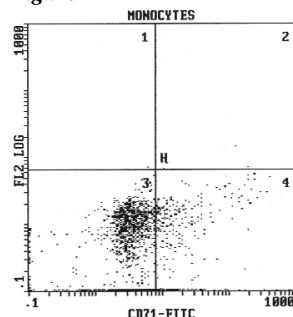


Figure C-1

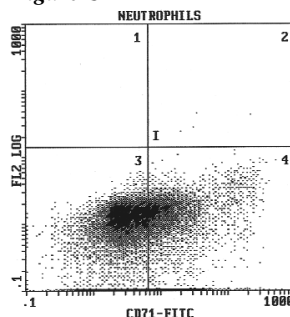


Figure A-2

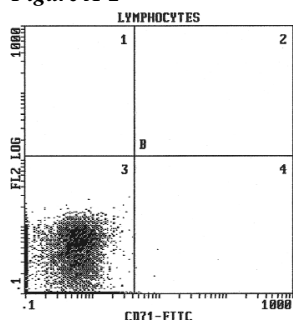


Figure B-2

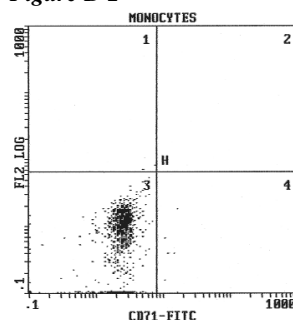
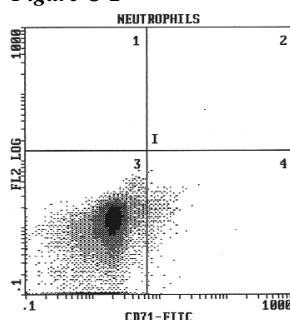


Figure C-2



General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.025 ml
# Particles	1.00 x 10 ⁷ per test
# Target Cells	4.69 x 10 ⁵ per test
Bead:Cell Ratio	21.3
% Depletion	97.43%

General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.025 ml
# Particles	1.00 x 10 ⁷ per test
# Target Cells	1.87 x 10 ⁵ per test
Bead:Cell Ratio	53.4
% Depletion	97.27%

General Recommendation*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.050 ml
# Particles	2.00 x 10 ⁷ per test
# Target Cells	2.92 x 10 ⁶ per test
Bead:Cell Ratio	6.8
% Depletion	96.52%

*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 CD71 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 A1 depicts the lymphocyte cell population prior to positive selection. Figure A2 depicts the lymphocyte cell population after positive selection. Figure B1 and B2 depict the results for monocytes, figure C1 and C2 neutrophils. The particle to cell ratios reported above are based on experiments where cells were exposed to the particles once.

Ordering Information:

Cat. #	Description	Size
85071	BioMag Anti-CD71 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

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