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BEADS ABOVE THE REST™

Description

The study of CD4 mediated events is an area of active research, specifically with regards to their role in autoimmune disorders, most notably those relating to Human Immunodeficiency Virus (HIV), as CD4 is a receptor for the Human Immunodeficiency Virus Type1 (HIV-1) envelope protein gp120. The CD4 antigen is expressed on a subset of peripheral blood lymphocytes, the T “helper” lymphocytes. It is also present on most thymocytes and is frequently co-expressed with CD8. CD4 is also expressed on all monocytes, but at a lower density than on CD4+ T lymphocytes. CD4+ T lymphocytes are active in inducing and helping the synthesis of immunoglobulins by B cells. Autoimmune disorders have been shown to be associated with T cell subset abnormalities due to a loss of inducer cells or due to the presence of abnormal T cells.

The CD4 antigen is a single-chain transmembrane glycoprotein with a 59 kDa molecular weight. It binds to a non-polymorphic region of MHC Class II molecules. CD4 is a co-receptor in MHC Class II restricted antigen-induced activation.

BioMag anti-Human CD4 particles are designed for positive selection of T helper lymphocytes and monocytes. The anti-CD4 MT310 clone used with BioMag recognizes human T helper lymphocytes and monocytes. BioMag anti-Human CD4 particles may be used in the detection of T cell acute lymphoblastic leukemia and lymphoblastic lymphoma and in the identification of T cell chronic lymphocytic leukemia derived from a helper population.

BioMag anti-Human CD4 is a suspension of magnetic particles approximately 1.5µm in size, which are coated with a mouse anti-human T cell IgG1 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

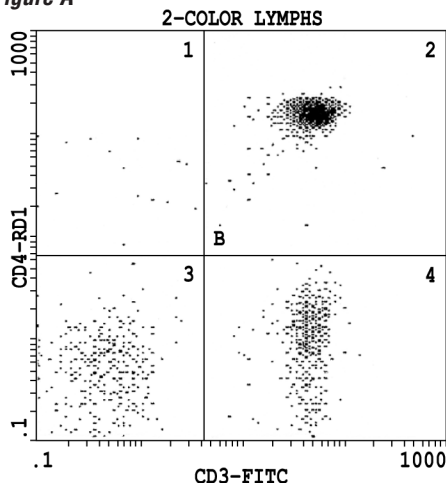
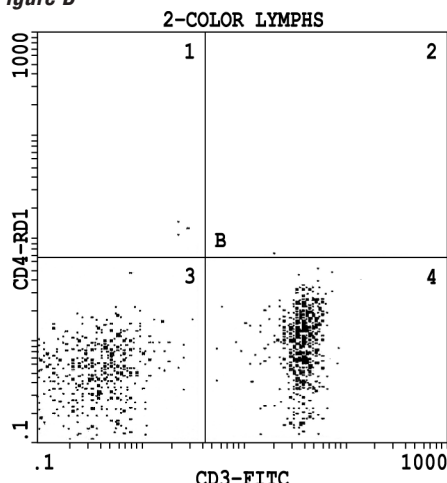


Figure B



General Recommendation*:

Concentration #	1.40 x 10 ⁸ particles/mL
Volume Used	0.018mL
# Particles	2.52 x 10 ⁶ per test
# Target Cells	6.77 x 10 ⁵ per test
Particles:Target Cell Ratio	3.7
% Depletion	99.44%

* 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 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.



Characteristics

Mean Diameter: ~1.5µm
 Particle Concentration: 1.5mg/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 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.

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
BM581	BioMag® anti-Human CD4	5mL

Order online anytime at www.bangslabs.com.