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B E A D S • A B O V E T H E R E S T™

DESCRIPTION

The CD11b antigen is the integrin alpha M-chain that combines with the CD18 antigen (integrin beta2) to form the CD11b/MAC-1 heterodimer. The CD11b/MAC-1 complex plays a role in cell-cell and cell-substrate interactions, and acts as a ligand for iC3b, CD50 (ICAM-3), and CD102 (ICAM-2), which is the CR3 complement receptor. CD11b is expressed by activated lymphocytes, granulocytes, and monocytes/macrophages. It is also weakly expressed by peripheral blood NK lymphocytes and a subset of T cells. In normal skin and tonsil tissue, expression of CD11b is restricted to macrophages.

BioMag® anti-Human CD11b particles are designed for positive selection of CD11b positive granulocytes and monocytes. It may also react with macrophages in normal tissue.

BioMag® anti-Human CD11b is a suspension of magnetic particles approximately 1.5µm in size. The suspension is supplied in a phosphate buffered saline (pH 7.5) containing EDTA, 1.0% BSA, and 0.1% sodium azide.

CHARACTERISTICS

Mean Diameter: ~1.5µm
 Particle Concentration: 1 mg/mL
 Particle Count: 1 x 10⁹ BioMag® particles per mg

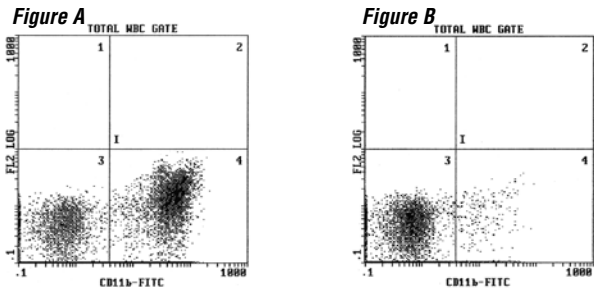
PROCEDURE

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.

Cell sorting results using BioMag® anti-Human CD11b 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.



General Recommendation*:

Concentration#	1.00 x 10 ⁸ particles/mL
Volume Used	50µL
# Particles	5.5 x 10 ⁶ per test
# Target Cells	1.73 x 10 ⁵ per mL
Particle:Target Cell Ratio	3:2
% Depletion	82.7%

*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.

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 CD11b particles in sterile media to remove preservative prior to use is recommended. Using a magnetic separation unit for washing instead of centrifugation is also 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.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
BM595	BioMag® anti-Human CD2	5mL

Order online anytime at www.bangslabs.com.