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B E A D S • A B O V E T H E R E S T [™]

DESCRIPTION

The CD14 antigen is found on cells of myelomonocytic lineage and is strongly expressed on monocytes and macrophages. The CD14 marker is useful for the detection and enumeration of adherent monocytes in normal peripheral blood and in disease states, such as the identification of leukemia and lymphoma cells of monocytic and myelomonocytic origin. It is present on blast cells from patients with myelomonocytic leukemia, but is infrequently expressed by cells from patients with acute granulocytic leukemia. CD14 is also found on Langerhans cells, follicular dendritic cells, and histiocytes, and is weakly expressed on B lymphocytes and neutrophils, but not expressed on T lymphocytes, NK cells, red blood cells, and platelets.

The CD14 antigen is a glycosyl-phosphatidylinositol-linked single-chain surface membrane glycoprotein with a molecular weight of 53-55 kDa and functions as a high affinity receptor for the complex of LipoPolySaccharides (LPS) and the LPS-Binding Protein. The CD14 antibody clone RM052 recognizes the Mo2 human myeloid antigen. It should be noted that the Mo2 antigen is not found on tumor cells isolated from patients with non-T acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and B and T cell non-Hodgkins lymphomas.

BioMag[®] anti-Human CD14 particles are designed for positive selection of adherent monocytes found in peripheral blood.

BioMag[®] anti-Human CD14 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: ~1.5 mg/mL
 Particle Count: 1 x 10⁸ BioMag[®] particles per mg

PROCEDURE

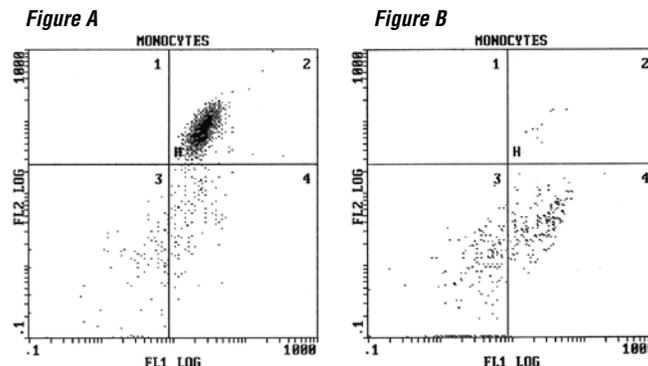
Researchers are advised to optimize the use of particles in any application.

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.

Cell sorting results using BioMag[®] anti-Human CD14 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# | 1.40 x 10 ⁸ particles/mL |
| Volume Used | 0.07mL |
| # Particles | 9.80 x 10 ⁶ per test |
| # Target Cells | 2.93 x 10 ⁵ per test |
| Particle:Target Cell Ratio | 33.5 |
| % Depletion | 96.90% |

** 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 CD14 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 |
|------------------|-------------------------|-------------|
| BM584 | BioMag® anti-Human CD14 | 5mL |

Order online anytime at www.bangslabs.com.