Bangs Laboratories, Inc.
BEADS ABOVE THE REST

BioMag® Human T cell Enrichment Systems

Product Data Sheet 597

DESCRIPTION

T cells are distinguished from other white blood cells by the presence of the T cell antigen receptor (TCR). Two types of TCR are routinely discussed, TCR-1 and TCR-2. TCR-1 is a disulfide-linked polypeptide containing γ and δ chains, whereas TCR-2 consists of α and β chains. Both of these receptors are associated with other polypeptides that make up the CD3 complex. Approximately 95% of T cells express TCR-2. The TCR-2 cells can be further subdivided into T helper CD4+ cells and T cytotoxic / suppressor CD8+ cells. The CD4+ cells recognize antigens in conjunction with MHC II class molecules, whereas CD8+ cells recognize antigens in conjunction with MHC class I molecules. CD3+ / TCR-1+ cells that are CD4- and CD8- are known as intra epithelial lymphocytes that target surface epithelia. In interstitial mucosal epithelium, TCR-1+ cells may also express CD8 and it is thought that these cells may operate at the site of pathogen entry through the epithelia.

The CD3 antigen is expressed by thymocytes and mature T cells. It is also present in the cytoplasm of the early thymocytes and has been utilized as an early marker of T cell differentiation. The CD4 antigen is expressed on a subset of peripheral blood lymphocytes, the T "helper" lymphocytes, 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 CD8 molecule is found on the suppressor / cytotoxic T lymphocytes, a subset of human peripheral blood lymphocytes, and is widely used as a marker of this cell type. Certain NK cells may also express the CD8 antigen, but with low to medium density of expression. CD8 is also present on most thymocytes where it is frequently co-expressed with CD4, and on a subpopulation of bone marrow cells. Many autoimmune diseases have been associated with a decrease in CD8 positive suppressor T lymphocytes.

The BioMag® Human T cell Enrichment Systems allow for the isolation of untouched CD3+, CD4+, and CD8+ T cells from human peripheral blood mononuclear cells. Based on BioMag® superparamagnetic particles, the BioMag® Human T cell Enrichment Systems target the removal of cells with a simple negative selection procedure that does not require the use of columns. The CD3+ system targets the removal of CD19, CD16, CD11b, CD56, and CD36 via antibodies attached to the BioMag® particles, resulting in over 95% purity of the CD3+ cells in the lymphocyte fraction. Using the BioMag® T cell CD4+ Enrichment System or the BioMag® Human T cell CD8+ Enrichment System facilitates the enrichment of CD4+ T cells or CD8+ T cells.

Each BioMag® Human T cell Enrichment System contains magnetic particles, which are 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

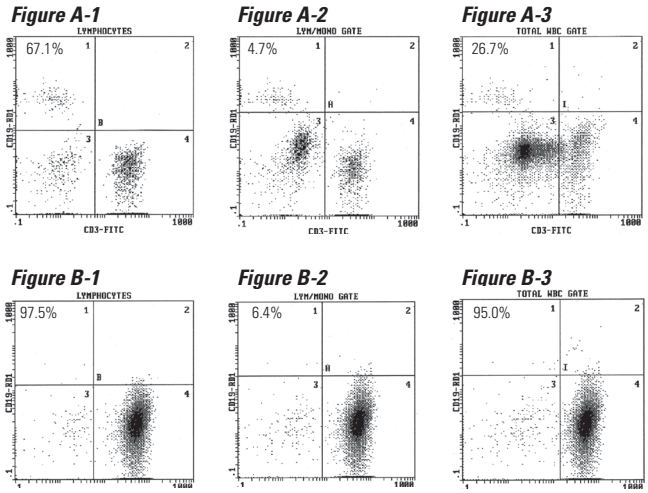
Mean Diameter: ~1.5µm
Particle Concentration: 1 mg/mL
Particle Count: 1 x 10^8 BioMag® particles per mg

RESULTS

Cell sorting results using BioMag® Human T cell Enrichment Systems are shown below. Typically, purified leukocytes and particles are incubated for 20-30 minutes and then magnetically separated. The supernatant is collected, incubated with the appropriate two-color antibody cocktail, and then analyzed by flow cytometry. The particle to cell ratios reported are based on experiments where the cells were exposed to the particles once.

Note: The values in the Purity Before and After Enrichment Charts 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 with BioMag® Human CD3+ T cell Enrichment Systems.
Figures A-1 through A-3 depict the cell populations prior to enrichment. Figures B-1 through B-3 depict the cell populations after enrichment.



Cell Type	Purity Before Enrichment	Purity After Enrichment
Lymphocytes	67.1%	97.5%
Lymphocytes, Monocytes	44.7%	96.4%
Lymphocytes, Monocytes, Neutrophils	26.7%	95.0%

The BioMag® Human CD3+ T cell Enrichment System targets removal of CD19+, CD16+, CD14+, CD56+, CD11b+, and CD36+ cells. The particle to target cell ratio is 5.6.

MATERIAL

Material Supplied

BioMag® T cell Enrichment particles, 1mL or 5mL

Material Required

Phosphate buffered saline (PBS	Test tubes
Ficoll	Centrifuge
Whole blood	Magnetic separator

PROCEDURE

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

Depending on the 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 or at lower temperatures, even as low as 4°C; however, incubation times should be extended at lower temperatures. A general procedure for T cell enrichment follows.

Cell Enrichment Protocol

1. Mix 5mL of Ficoll with 5mL of whole blood.
2. Centrifuge at 400 x g for 30 minutes.
3. Isolate the mononuclear fraction from the interface and place in a fresh tube.
4. Bring the mononuclear fraction to 10mL with PBS. Gently mix and then centrifuge at 300 x g for 10 minutes.
5. Carefully remove the supernatant, resuspend the cell pellet in 10mL of PBS, and centrifuge at 300 x g for 10 minutes.
6. Carefully remove the supernatant and resuspend the pellet in 5mL (original blood volume) of PBS.
7. Count the cells and adjust the concentration to 2–5 x 10^6/mL in PBS.
8. Wash 50-100µL of the BioMag® suspension by magnetically separating the particles, removing the supernatant, and resuspending the particles in an equal volume of PBS.
9. Add 1mL of the mononuclear fraction to the washed BioMag® particles, mix, and incubate for 20-30 minutes. *Note:* Incubation temperature can vary. Incubation at 4°C is recommended for most applications. Incubation at room temperature has also produced good results.
10. Magnetically separate until the supernatant is relatively clear (3-5 minutes); collect the supernatant to a fresh tube. This is the enriched fraction.
11. Test the enriched cell population by flow cytometry after staining the cells with the appropriate labeled antibody cocktail. A suggestion would be to test the T cell enriched fraction by staining with anti-CD3FITC and anti-CD19RD1.

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® T cell Enrichment 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

These particle suspensions contain 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 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	Sizes
BM597	BioMag® Human CD3+ T cell Enrichment System	1mL or 5mL
BM598	BioMag® Human CD4+ T cell Enrichment System	1mL or 5mL

Order online anytime at **www.bangslabs.com.**

Cell sorting results with BioMag® Human CD4+ T cell Enrichment Systems.
Figures A-1 through A-3 depict the cell populations prior to enrichment. Figures B-1 through B-3 depict the cell populations after enrichment.

Figure A-1 ***Figure A-2*** ***Figure A-3***

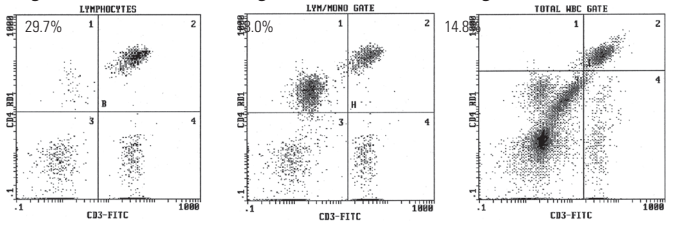
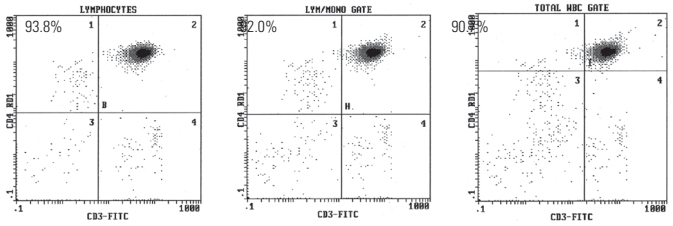


Figure B-1 ***Figure B-2*** ***Figure B-3***



Cell Type	Purity Before Enrichment	Purity After Enrichment
Lymphocytes	29.7%	93.8%
Lymphocytes, Monocytes	18.0%	92.0%
Lymphocytes, Monocytes, Neutrophils	14.8%	90.7%

The BioMag® Human CD4+ T cell Enrichment System targets removal of CD19+, CD16+, CD14+, CD56+, CD11b+, CD36+, and CD8+ cells. The particle to target cell ratio is 6.0.

Cell sorting results with BioMag® Human CD8+ T cell Enrichment Systems.
Figures A-1 through A-3 depict the cell populations prior to enrichment. Figures B-1 through B-3 depict the cell populations after enrichment.

Figure A-1 ***Figure A-2*** ***Figure A-3***

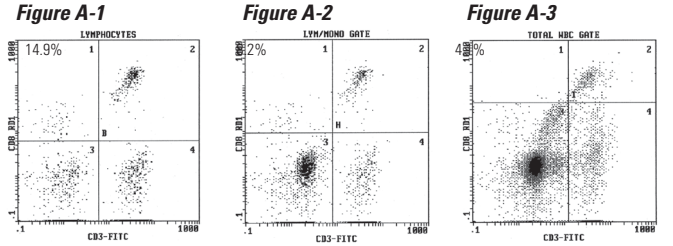
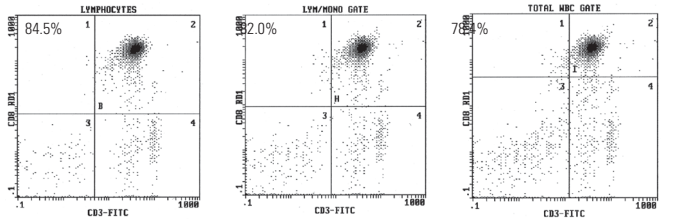


Figure B-1 ***Figure B-2*** ***Figure B-3***



Cell Type	Purity Before Enrichment	Purity After Enrichment
Lymphocytes	14.9%	84.5%
Lymphocytes, Monocytes	8.2%	82.0%
Lymphocytes, Monocytes, Neutrophils	4.1%	78.4%

The BioMag® Human CD8+ T cell Enrichment System targets removal of CD19+, CD16+, CD14+, CD56+, CD11b+, CD36+, and CD4+ cells. The particle to target cell ratio is 6.0.