

Human Immunology Center Core Laboratory
David H. Smith Center for Vaccine Biology and Immunology
Aab Institute of Biomedical Sciences

STANDARD OPERATING PROCEDURE: Negative or Positive Selection of a Specific Cell Population from Human Peripheral Blood Mononuclear Cells Utilizing Magnetic Cell Sorting Kit from Miltenyi Biotec.

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Author: Shelley Secor-Socha Approval:

1. Purpose:

The isolation of a specific cell population using MACS Magnetic cell sorting technology for labeling the cell population of interest (Positive Selection). The use of the MACS Magnetic cell sorting technology for labeling all other cell populations except for the cell population of interest leaving an untouched cell population (Negative Selection).

2. Principle:

The principle for each specific cell population isolation procedure can be found in the individual protocol that is included with the reagent. For positive selection, the cell population of interest is labeled with MACS microbeads for 15 minutes at 4°C. The cells are then placed into a MACS column that is placed in a magnetic field. The labeled cells are retained on the column while in the magnetic field while the unlabeled cells flow through the column. The column is then removed from the magnetic field and the labeled cells are eluted from the column. Generally for negative selection of a target cell population, unwanted cells are labeled with a monoclonal antibody complex conjugated to biotin for 10 minutes at 4°C. The cells are then labeled with an anti-biotin monoclonal antibody conjugated to microbeads for 15 minutes at 4°C. The excess reagents are washed away and the cells are placed in a MACS column within a magnetic field. The labeled cells adhere to the MACS column while the unlabeled population of interest pass through the column and are collected. If needed the labeled cells may be eluted after removing the column from the magnetic field.

3. Reference:

For each specific isolation, reference the product insert.

4. Materials and Reagents:

Human peripheral blood mononuclear cells
Specific Magnetic Cell Sorting Kit from Miltenyi Biotec
MS Column, Miltenyi Biotec, Cat # 130-042-201
LS Column, Miltenyi Biotec, Cat # 130-042-401
LD Columns, Miltenyi Biotec, Cat # 130-042-901
Magnets: QuadroMACS or OctoMACS
Hanks Balanced Salt Solution, Cellgro, Cat # 21-022-CV

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Bovine Serum Albumin(BSA), Bovuminar® Reagent Pure pH 7.0, Intergen, Cat # 3160-60
Finnpipette 5-50µL single channel pipet, Thermo Electron, Cat # 4600220
Finnpipette 1-10µL single channel pipet, Thermo Electron, Cat # 4600180
Finnpipette 30-300µL single channel pipet, Thermo Electron, Cat # 4600240
Finnpipette 100-1000µL single channel pipet, Thermo Electron, Catalog# 4600250
Sterile 50-1000µL disposable pipet tips, Eppendorf, Catalog# 2249194-6
Sterile 20-200µL disposable pipet tips, Eppendorf, Catalog # 2249193-8
Sterile 30-300µL disposable pipet tips, Eppendorf, Catalog # 2249194-6
Sterile 0.1-20µL disposable pipet tips, Eppendorf, Catalog # 2249113-0
1 mL sterile disposable serological pipets, Costar, Catalog # 4012
5 mL sterile disposable serological pipets, Costar, Catalog # 4051
10 mL sterile disposable serological pipets, Costar, Catalog # 4101
25 mL sterile disposable serological pipets, Costar, Catalog # 4251
Pipet Aid, Drummond, Catalog # 4-000-101
15 mL sterile polypropylene conical tubes, Falcon, Catalog # 35-2096
50 mL sterile polypropylene conical tubes, Falcon, Catalog # 35-2070
12x75mm culture tubes with closures, VWR Cat # 60818-500
Household bleach for decontamination, VWR, Catalog # 37001-056
Biological safety cabinet, Class II, Baker, Model # SG403
10% hypochlorite solution
Distilled water
Pre-separation Filter, Miltenyi Biotec, Catalog# 130-041-407
Centrifuge, Beckman Coulter, Allegra X-12R or equivalent
Trypan Blue Solution (0.4%) Sigma Catalog# T8154
EDTA (Ethylenediaminetetraacetic acid tetrasodium salt), Sigma, Catalog# E6511

4.1. Reagent preparation:

- 4.1.1. Separation Buffer: Hanks Balanced Salt Solution (HBSS)/0.5%BSA/2mM EDTA:
Add 5 g BSA and 0.8g EDTA to 1 liter of HBSS. Let BSA and EDTA dissolve. Sterile filter using a 0.45-micron filter into a sterile bottle. Degas. Store at 4°C and keep sterile.

5. Procedure:

5.1. Prepare fresh or frozen Peripheral blood mononuclear cells as described below:

- 5.1.1. PBMC are isolated from fresh blood using ficoll hypaque separation or BD CPT tubes and spun twice at 300xg for 10 minutes to remove platelets.

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5.1.2. PBMC frozen in LN₂ are rapidly thawed with shaking in 37° C water bath until last ice crystal remains. The cells are immediately added to an empty 15mL tube, and then cold HBSS/1%BSA is added drop wise with gentle mixing. After approximately 2mls of HBSS/1%BSA has been added, the rate of addition can be increased. The diluted cell suspension is spun at 300xg for 10 minutes at 4° C to remove DMSO. The supernatant is removed by pouring it into a waste container through gauze and into a disinfectant solution. Tap tubes gently with finger to resuspend cells and repeat wash once with 10mL of cold HBSS/1%BSA. Do not vortex or rub tube roughly along a tube rack. Resuspend cells in 5mL HBSS/0.5%BSA/2mM EDTA. Prepare a 1:10 dilution with 0.4 % trypan blue exclusion dye in saline (i.e. 5 µL into 45 µL). Count cells and record number.

5.2. Follow the product insert for the positive or negative selection of the specific cell population of interest. The insert is included in the product box and is also available online at www.miltenyibiotec.com

Notes

1. All incubation are performed a 4°C with cold buffer. Higher temperatures or longer incubation times than recommended may lead to non-specific cell labeling
2. Verify the correct column is being used. Columns have a limitation for the total number of cells being used and also the total number of cells being labeled. See the chart below.
3. It is recommended that the cell suspension be filtered before separating the cells to ensure a single-cell suspension.
4. The volumes provided in the product insert are for 10⁷ total cells. Increase volumes if more cells are being used. For less than 10⁷ cells do NOT decrease the volumes, use the volumes for 10⁷ cells.
5. PBS/0.5%BSA/2mM EDTA is the buffer recommended by Miltenyi Biotec in the Product insert. This procedure uses HBSS/1%BSA. Either reagent is acceptable for use.

Column Type	Maximum number of labeled cells	Maximum number of total cells
MS	10 ⁷	2 x 10 ⁸
LS	10 ⁸	2 x 10 ⁹
LD	10 ⁸	5 x 10 ⁸

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Revision History

Version	Change	Impact	Justification	Change Date:
HIC-1-0013	New		Control document management system	1/4/07