Monoclonal Anti-mouse CD8α Antibody

ORDERING INFORMATION

Catalog Number: MLDP4

Clone: 53-6.7

Lot Number: FSG03

Size: 1 vial

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS with 0.1% BSA

Specificity: mouse CD8α

Immunogen: mouse thymus or spleen

Ig class: Rat IgG_{2A}

Application: $CD8\alpha^+$ Cell Depletion

Preparation
This antibody was produced from a murine hybridoma elicited from a rat immunized with mouse thymus or spleen.[1] The IgG fraction of tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with 125 μL of sterile PBS containing 0.1% BSA, very low endotoxin (Serologicals Proteins Inc. Catalog # 81-068-3 or equivalent).

Storage
Lyophilized samples are stable for greater than six months when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for at least 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for at least six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody has been shown to react with both the α and α′ chains of mouse CD8 (Ly-2 or Lyt-2), an antigen co-receptor on the T cell surface which interacts with MHC I molecules on antigen presenting cells.[2] CD8αβ heterodimer is expressed on a subpopulation of mature T cells.[3,4] CD8α, without CD8β, has been detected on subsets of γδ TCR-bearing T cells,[4] intestinal intrathymic lymphocytes[6,7] and dendritic cells.[8,9]

Applications
Lineage Depletion - This antibody has been used in combination with antibodies to other lineage markers to deplete the cells of major hematopoietic lineages. Each vial contains sufficient antibody to deplete $CD8^+$ cells from 1 x 10^9 bone marrow cells.

References:

1. Ledbetter, J.A. and L.A. Herzenberg, 1979, Immunol. Rev. **47**:63 - 90
2. Bierer, B.E. *et al*., 1989, Annu. Rev. Immunol. **7**:579 - 599.
3. Ledbetter, J.A. *et al*., 1980, J. Exp. Med. **152**:280 - 295.
4. Hayakawa, K. *et al*., 1994, Science **263**:1131 - 1133.
5. MacDonald, H.R. *et al*., 1990, Eur. J. Immunol. **20**:927 - 930.
6. Rocha, B. *et al*., 1992, Immunol. Today **13**:449 - 454.
7. Wang, J. and J.R. Klein, 1994, Science **265**:1860 - 1862.
8. Vermec, D. *et al*., 1992, J. Exp. Med. **176**: 47 - 58.
9. Suss, G. and K. Shortman, 1996, J. Exp. Med. **183**:1789 - 1796.

R&D Systems, Inc.
1-800-343-7475

Lineage Depletion Protocol

Monoclonal anti-mouse CD3, CD4, CD5, CD8α, CD11b/MAC-1α, B220, Gr-1 (Ly-6G) and TER-119 erythroid antigen antibodies can be used to efficiently stain bone marrow-derived cells committed to major hematopoietic lineages, including T lymphocytes, B lymphocytes, monocytes/macrophages, granulocytes and erythrocytes. These antibodies can be used in conjunction with magnetic particle separation systems or flow cytometric cell sorting to deplete lineage-committed cells for the enrichment of mouse uncommitted hematopoietic progenitors.

Supplies Required

Materials

- Mice, 6 - 8 weeks old
- 50 mL centrifuge tubes (Falcon Catalog # 352098 or equivalent)
- 15 mL centrifuge tubes (Corning Costar Catalog # 430052 or equivalent)
- 0.2 μm, 500 mL filter units (Nalgene Catalog # 161-0020 or equivalent)
- 0.2 μm syringe filters (PALL Corporation Part # 4187 or equivalent)
- 10 mL syringes (Becton Dickinson Catalog # 309604 or equivalent)
- 70 μm cell strainers (Falcon Catalog # 352350 or equivalent)
- 5 mL polypropylene tubes, 12 x 75 mm with snapcaps (Falcon Catalog # 352063 or equivalent)
- 2 mL polypropylene microtubes (Sarstedt Catalog # 72694.006 or equivalent)
- Scalpel blades
- 21-gauge needles
- 23-gauge needles
- 25-gauge needles
- Serological pipettes
- Pipettors and pipette tips

Reagents

- Monoclonal anti-mouse CD3, CD4, CD5, CD8α, CD11b/MAC-1α, B220, Gr-1 (Ly-6G) and TER-119 erythroid antigen antibodies (R&D Systems, Catalog # MLDP1, MLDP2, MLDP3, MLDP4, MLDP5, MLDP7, MLDP6 and MLDP8, respectively)
- Phosphate-Buffered Saline (PBS) (GIBCO Catalog # 10010-023 or equivalent)
- BSA, very low endotoxin (Serologicals Proteins Inc. Catalog # 81-068-3 or equivalent)
- Fetal Bovine Serum (FBS), ES Cell Qualified (GIBCO Catalog # 16141-061 or equivalent)
- Sterile, deionized water
- Mouse Erythrocyte Lysing Kit (R&D Systems, Catalog # WL2000 or equivalent)
- Dynabeads® M-450 Sheep anti-rat IgG (Dynal Biotech Product # 110.07 or equivalent)
- Anti-rat IgG-FITC (Caltag Catalog # R40101 or equivalent) or anti-rat IgG-PE (Caltag Catalog # R40104 or equivalent)

Equipment

- 4° C Centrifuge
- Rotator
- Hemocytometer
- Microscope
- Magnetic particle concentrator (Dynal Biotech Product # 120.21 or equivalent)

Reagent Preparation

Depletion Antibody Cocktail - Mix 12.5 μL of each of the monoclonal anti-mouse CD3, CD4, CD5, CD8α, CD11b/MAC-1α, B220, Gr-1 (Ly-6G) and TER-119 erythroid antigen antibodies in a 2 mL polypropylene microtube to make 100 μL of antibody cocktail (sufficient to deplete lineage positive cells from 1 x 10^8 bone marrow cells). Mix gently. Prepare fresh as needed.

PBS with 2% FBS - Add 10 mL of FBS to 490 mL of PBS to make 500 mL of PBS with 2% FBS. Sterile filter the solution using a 0.2 μm, 500 mL filter unit. Store at 2 - 8° C for up to 1 month.

M-lyse Buffer (optional) - Add 1 mL of 10X M-lyse Buffer (contained in the Mouse Erythrocyte Lysing Kit) to 9 mL of sterile, distilled water. Store at 2 - 8° C for up to one year.

Preparation of Bone Marrow Cells

Use sterile technique if the cells are to be used for* in vitro *culture or* in vivo *assays after depletion.

1. Harvest the hind leg bones and collect the bone marrow cells of mice. For a detailed protocol, please refer to reference 1.
2. Centrifuge the cells for 5 minutes at 400 x g and 4° C. Remove the supernate and resuspend the cells in 10 mL of cold PBS with 2% FBS by gentle pipetting, to generate a single cell suspension. Filter the cell suspension through a 70 μm cell strainer to remove cell clumps.
3. After filtration, the cells can be used directly for depletion. Or, if desired, the red blood cells (RBC) can be removed. To remove RBC, resuspend the cells in 5 mL of M-lyse Buffer followed by centrifugation for 5 minutes at 400 x g and 4° C. Remove the supernate and resuspend the cells in cold PBS with 2% FBS. Count the viable cells. After RBC lysis, one mouse typically yields 30 - 60 x 10^6 cells.

Lineage Depletion of Bone Marrow Cells
Followed by negative selection with Dynabeads®

1. After the viable cell number is determined, resuspend the cells in cold PBS with 2% FBS to a final density of 1 x 10^8 cells/400 μL. Transfer the cells to a 5 mL polypropylene tube. Retain approximately 0.5 x 10^6 cells to stain with anti-rat IgG-FITC or -PE as a control.
2. Add 100 μL of Depletion Antibody Cocktail to each 400 μL of cells. Incubate the cells on ice for 40 minutes. Mix the cells every 10 minutes.
3. Wash the cells 3 times with 3 mL of cold PBS with 2% FBS to remove excess antibody by centrifugation at 400 x g for 10 minutes. Retain approximately 0.5 x 10^6 cells to stain with a suitable anti-rat IgG-FITC or -PE conjugate to assess the proportion of lineage positive cells. Compare this sample with the cell preparation at the end of the procedure to determine the depletion efficiency.
4. Remove the supernate by pipetting and suspend the cells in 1 mL of cold PBS with 2% FBS.
5. Prepare Dynabeads M-450 Sheep anti-rat IgG by calculating the volume of beads needed based on a bead to cell ratio of 2:1. Wash the appropriate volume of beads twice with 3 mL of cold PBS with 2% FBS, capturing the beads with a magnet after each wash. Resuspend the beads in 200 μL PBS with 2% FBS.
6. Add 100 μL of washed beads to the cells from step 4. Incubate on ice bath for 5 minutes with constant hand agitation. Add cold PBS with 2% FBS to bring the volume up to 3 mL. Cap the tube and rotate end over end for 25 minutes at 2 - 8° C to facilitate cell binding.
7. Capture the lineage-positive cell-bound beads using a magnet for 2 minutes. Transfer the unbound, lineage-negative cells, using a sterile plastic pipette **(do not use a glass pipette)**, to a 5 mL polypropylene tube. Discard the beads. Centrifuge the lineage-negative cells for 5 minutes at 400 x g and 4° C
8. Discard the supernate and resuspend the lineage-negative cells in 1 mL cold PBS with 2% FBS. Add the remaining 100 μL of washed beads and incubate as in step 6.
9. Capture the residual lineage-positive cells and harvest the lineage-negative cells as in step 7. Count the viable cells. This procedure removes approximately 98 - 99% of the lineage-positive cells.
10. Label a small aliquot of the lineage-negative cells with an appropriate dilution of anti-rat-IgG-FITC or -PE conjugated to determine the efficiency of lineage-positive cell removal. Compare the efficiency with that obtained from the aliquot of cells stained in the identical manner from step 3. See Figure 1 below for an example of lineage depletion using the depletion antibody cocktail. After depletion the lineage-negative cells contain enriched CD117 (SCF R/c-kit) cells (typical 70 – 90%) and less than 2% of lineage-positive contamination.

Figure 1

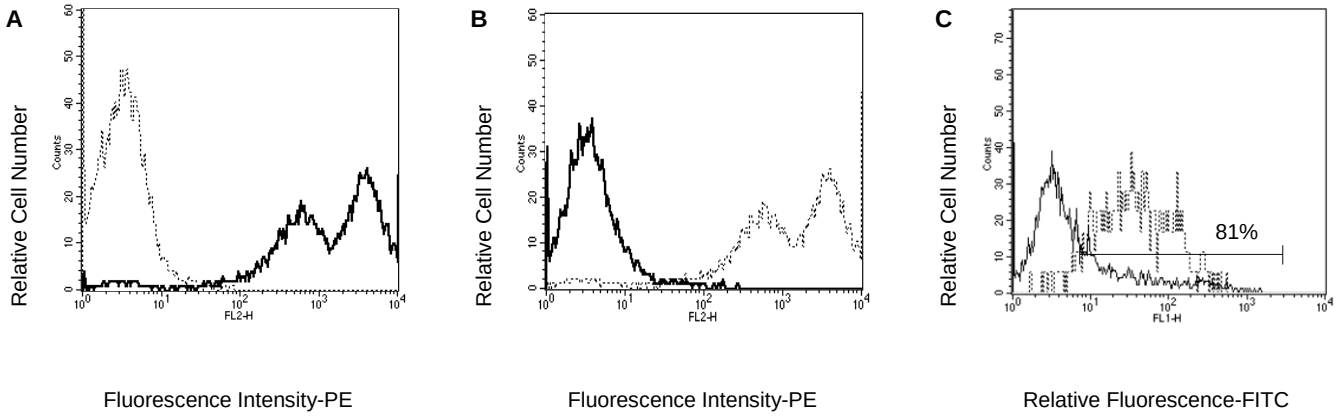

Figure 1: *Lineage positive bone marrow cells from C57/Bl6 mice were depleted using monoclonal anti-mouse CD3, CD4, CD5, CD8α, CD11b/MAC-1α, B220, Gr-1 (Ly-6G) and TER-119 erythroid antigen antibodies and stained with goat anti-rat IgG PE-conjugated. Flow cytometric analysis was performed **A)** Before (dotted histogram) and after (solid histogram) the depletion antibody cocktail was added; **B)** Before (dotted histogram) and after (solid histogram) magnetic depletion using Dynabeads; and **C)** Expression of CD117 (SCF R/c-kit) (R&D Systems, Catalog # MAB1356; dotted histogram) in bone marrow-progenitor cells after enrichment using the mouse lineage panel and bead depletion.*

Reference

1. *Current Protocols in Immunology*, *Isolation of Murine Macrophages*, copyright 1994, Coligan, J.E. *et al.* eds., John Wiley & Sons, Inc., Volume 3, Supplement 11, 14.1.4.