Technical Data Sheet

Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set - DM

Product Information

Material Number: 558451

Component: 51-9000794

Description: Biotin Mouse Lineage Depletion Cocktail

Size: 5.0 ml (1 ea)

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Component: 51-90008

Description: Streptavidin Particles Plus - DM

Size: 5.0 ml (1 ea)

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD IMagTM Mouse Hematopoietic Progenitor Cell Enrichment Set - DM reacts with cells from the major hematopoietic cell lineages, such as T lymphocytes, B lymphocytes, monocytes/macrophages, granulocytes, and erythrocytes. The Biotinylated Mouse Lineage Depletion Cocktail contains biotinylated monoclonal antibodies to mouse CD3e (CD3 ε chain), CD11b (Integrin αM chain), CD45R/B220, Ly-6G and Ly-6C (Gr-1), and TER-119/Erythroid Cells (Ly-76). The BD IMagTM Streptavidin Particles Plus - DM are magnetic nanoparticles that have streptavidin covalently conjugated to their surfaces. This Set is designed for the immunomagnetic enrichment of hematopoietic progenitors from mouse bone marrow by depletion of cells committed to the T- and B-lymphocytic, myeloid (monocytic and granulocytic), and erythroid lineages. The Set contains sufficient reagents to label 10^9 bone marrow cells.

Biotin Mouse Lineage Depletion Cocktail is comprised of the following biotin-conjugated monoclonal antibodies:

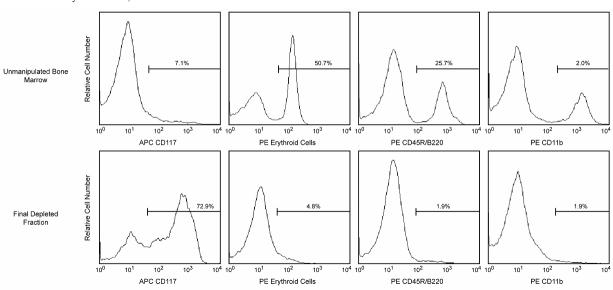
Anti-mouse CD3e, clone 145-2C11

Anti-mouse CD45R/B220, clone RA3-6B2

Anti-mouse TER-119/Erythroid Cells, clone TER-119

Anti-mouse CD11, clone M1/70

Anti-mouse Ly-6G and Ly-6C (Gr-1), clone RB6-8C5



Depletion of lineage-committed cells from mouse bone marrow. BALB/c bone-marrow cells were labeled with the BD IMag™ Mouse Hematopoietic Progenitor Enrichment Set and separated on the BD IMagnet™ (Cat. No. 552311) according to the accompanying Protocol. To demonstrate the efficiency of the depletion, unmanipulated bone marrow cells and the final depleted fraction were stained with APC-conjugated anti-mouse CD117 mAb 2B8 (Cat. No. 553356) to detect hematopoietic progenitors, and with PE-conjugated mAb TER-119 (Cat. No. 553673), PE-conjugated mAb RA3-6B2 (Cat. No. 553089/553090), and PE-conjugated mAb M1/70 (Cat. No. 557397/553311) to detect lineage-committed cells. The percentage of positive cells is indicated in each panel; placement of each marker is based upon staining with the appropriate isotype control (data not shown). The final depleted fraction contains a greatly increased proportion of CD117+ cells and less than 5% of lineage-positive contaminants.

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Preparation and Storage

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.

Store undiluted at 4°C.

Application Notes

Recommended Assay Procedure:

The detailed Magnetic Labeling and Depletion Protocol follows. In summary, the Biotinylated Mouse Lineage Depletion Cocktail simultaneously stains the lineage-committed hematopoietic cells according to their different specificities. After washing away excess antibody, BD IMagTM Streptavidin Particles Plus - DM are added to the cell suspension and bind the cells bearing the biotinylated antibodies. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnetTM (Cat. No. 552311). Negative selection is then performed to enrich for uncommitted hematopoietic progenitors. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off and retained (depleted fraction). Additional negative selections are performed to optimize the yield and purity of the depleted fraction. The magnetic separation steps are diagrammed in the Depletion Flow Chart. Both the positive and depleted fractions can be evaluated in downstream applications such as flow cytometry and tissue culture. The antibodies in the Biotinylated Mouse Lineage Depletion Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of bone marrow hematopoietic progenitors.

MAGNETIC LABELING AND DEPLETION PROTOCOL

- 1. Prepare sterile buffers and place on ice.
 - a. Cell-staining buffer: Phosphate Buffered Saline supplemented with 3% heat-inactivated fetal calf serum and 0.1% sodium azide
 - b. 1X BD IMagTM buffer: Dilute BD IMagTM Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.
- Aseptically prepare a single-cell suspension from mouse bone marrow. Remove clumps of cells and/or debris by passing the suspended cells through a 70-um nylon cell strainer.
 - Note: The femurs and tibiae of one adult mouse typically yield 20-60 x 10⁶ hematopoietic cells. One mouse will yield approximately 0.3-1.0 x 10⁶ lineage-negative cells.
- 3. Count the cells and resuspend them in sterile cell-staining buffer at 10 to 20 x 10⁶ cells/ml. Set aside a sample of unstained cells (~5 x 10⁶ cells) to be used in the flow cytometric analysis in Step 17.
- Add Mouse BD Fc BlockTM purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) at 0.25 μg/10⁶ cells, and incubate on ice for 15 minutes.
- 5. Add the Biotinylated Mouse Lineage Depletion Cocktail at 5 µl per 1 x 106 cells, and incubate on ice for 15 minutes.
- 6. Wash the labeled cells with a 10X excess volume of 1X BD IMag[™] buffer, centrifuge at 300 x g for 7 minutes, and carefully aspirate ALL the supernatant.
- 7. Vortex the BD IMag™ Streptavidin Particles Plus DM thoroughly, and add 5 µl of particles for every 1 x 10^6 total cells.
- 8. MIX THOROUGHLY. Refrigerate for 30 minutes at 6°C 12°C.
- 9. Bring the labeling volume up to 20 to 80 x 10⁶ cells/ml with 1X BD IMag[™] buffer.
- 10. Transfer the labeled cells to a 12 x 75 mm round-bottom test tube (eg, BD FalconTM, Cat. No. 352058), maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMagnetTM (horizontal position) for 8 minutes.
 - For greater volume, transfer the cells to a 17 x 100 mm round-bottom test tube (eg, BD Falcon™, Cat. No. 352057), maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMagnet (vertical position) for 10 minutes.
- 11. With the tube on the BD IMagnet[™] and using a sterile glass Pasteur pipette, carefully aspirate the supernatant (depleted
 - fraction) and place in a new sterile tube.
- 12. Remove the positive-fraction tube from the BD IMagnet[™], and add 1X BD IMag[™] buffer to the same volume as in Step 9. Resuspend the positive fraction well by pipetting up and down 10 to 15 times, and place the tube back on the BD IMagnet[™] for 8 minutes.
 - 17 x 100 mm tube: Place on the BD IMagnet™ for 10 minutes.
- 13. Using a new sterile Pasteur pipette, carefully aspirate the supernatant and combine with the depleted fraction from Step 11 above.
- 14. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry, if desired.
- 15. Place the tube containing the combined depleted fraction on the BD IMagnetTM for a final 8 minutes.
 - 17 x 100 mm tube: Place on the BD IMagnet™ for 10 minutes.
- 16. Carefully aspirate the supernatant and place in a new sterile tube. This is the final depleted fraction containing enriched hematopoietic progenitors. The cells are ready to be processed for downstream applications.
- 17. Samples of the total cell suspension and the positive and final depleted fractions should be analyzed by flow cytometry to evaluate the efficiency of the cell-separation procedure.

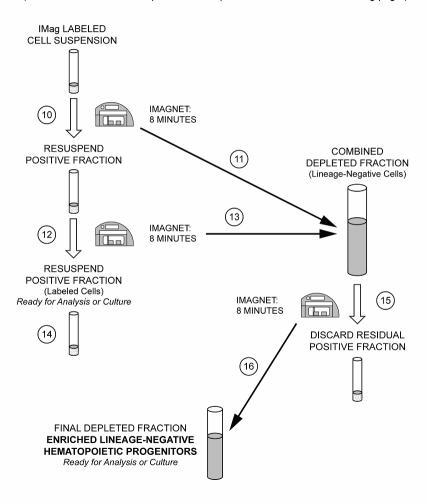
NOTES:

- After washing away excess biotinylated antibody, completely aspirate the supernatant. Supernatant left in the tube will increase the labeling volume, which will decrease the efficiency of magnetic labeling.
- When labeling cells with the BD IMag™ Streptavidin Particles Plus DM, use biotin-free buffer only. Free biotin will compete with the biotinylated antibody for binding to the BD IMag™ Streptavidin Particles Plus DM.
- Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

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DEPLETION FLOW CHART

(The circled numbers correspond to the steps of the Protocol on the following page.)



Product Notices

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- BD IMagTM particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology and are licensed under US patent number 7,169,618.
- 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Goodell MA, Rosenzweig M, Kim H, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med.* 1997; 3(12):1337-1345.(Biology)

Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. *Development.* 1997; 124(10):1929-1939.(Biology)

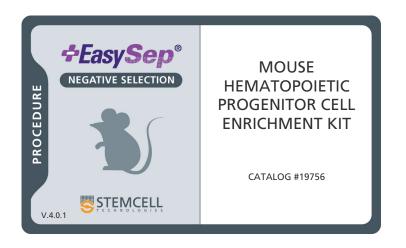
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Sato T, Laver JH, Ogawa M. Reversible expression of CD34 by murine hematopoietic stem cells. *Blood*. 1999; 94(8):2548-2554.(Biology)

Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988; 241(4861):58-62. (Biology)

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THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A), THE PURPLE EASYSEP® MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP® MAGNET (SECTION C).

A) FULLY AUTOMATED PROTOCOL USING ROBOSEP® (CATALOG #20000).

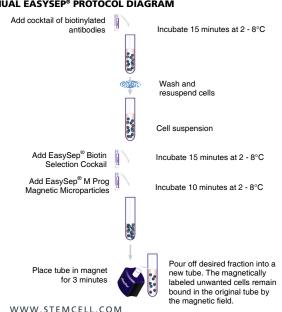
This procedure is used for processing 500 μ L - 8.0 mL of sample (up to 8.0 x 10⁸ cells).

- 1. Prepare nucleated cell suspension at a concentration of 1 x 10⁸ cells/mL in RoboSep[®] Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel. Add Normal Rat Serum (provided) at 50 μL per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of serum).
 - $\mathit{Falcon}^{\mathsf{TM}}$ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
- Add EasySep® Mouse Hematopoietic Progenitor Enrichment Cocktail at 50 μ L/mL of cells (e.g. for 2 mL of cells, add 100 μ L of cocktail). Mix well and incubate at 2 - 8°C for 15 minutes.
- 3. Wash cells and resuspend at 1 x 108 cells/mL in recommended medium.
- 4. Select the appropriate RoboSep® protocol:
 - · For high purity, select the protocol entitled "Mouse Progenitor Negative Selection 19756 - high purity".
 - For high recovery, select the protocol entitled "Mouse Progenitor Negative Selection 19756 - high recovery".

If a modified RoboSep® protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

- 5. Load the RoboSep® carousel as directed by the on-screen prompts. Vortex the EasySep® D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates. When all desired quadrants are loaded, press the green "Run" button. All remaining cell labeling and separation steps will be performed by RoboSep®
- When cell separation is complete, remove the enriched cells in the 50 mL tube located to the left of the tip rack. The enriched cells are now ready for use.

MANUAL EASYSEP® PROTOCOL DIAGRAM



B) MANUAL EASYSEP® PROTOCOL USING PURPLE EASYSEP® MAGNET (CATALOG #18000).

This procedure is used for processing 500 μ L – 2.0 mL of sample (up to 2 x 10⁸ cells)

- 1. Prepare single nucleated cell suspension at a concentration of 1 x 108 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the purple EasySep® Magnet. Add Normal Rat Serum (provided) at 50 μ L per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).
 - Falcon™ 5 mL (BD, Catalog #352058) Polystyrene Round-Bottom Tubes are recommended.
- 2. Add EasySep® Mouse Hematopoietic Progenitor Cell Enrichment Cocktail at 50 μL/mL of cells (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for 15 minutes
- 3. Wash cells and resuspend at 1 x 108 cells/mL in recommended medium.
 - Note. The wash step is recommended if a high depletion of lineage antigen positive cells is required and the start number of desired cells is low. However, if high recovery is more desirable than high purity, it is recommended to leave out the wash step.
- 4. Add EasySep® Biotin Selection Cocktail at 100 μL/mL of cells (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate at 2 - 8°C for 15 minutes.
- 5. Vortex EasySep® Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds to ensure that the particles are in a uniform suspension with no visible aggregates.
- 6. Add the magnetic particles at 50 μ L/mL of cells (e.g. for 2 mL of cells, add 100 μ L of magnetic particles). Mix well and incubate at 2 - 8°C for 10 minutes
 - Note: For increased depletion of lineage antigen positive cells, magnetic particles may be added at 75 µL/mL of cells. This will increase purity but will decrease recovery of
- 7. Bring the cell suspension to a total volume of 2.5 mL by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for 3 minutes.
- 8. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the magnet. Leave the magnet and the tube inverted for 2 - 3 seconds, then return to upright position. Do not shake or blot off any drops that may remain hanging from the mouth of the tube. The enriched cells in the new tube are now ready for use.

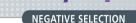
C) MANUAL EASYSEP® PROTOCOL USING "THE BIG EASY" SILVER EASYSEP® MAGNET (CATALOG #18001).

This procedure is used for processing 500 μL – 8.5 mL of sample (up to 8.5 x 10⁸ cells).

- 1. Prepare nucleated cell suspension at a concentration of 1 x 108 cells/mL in recommended medium (See Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the silver magnet. Add Normal Rat Serum (provided) at 50 μ L per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).
 - Falcon[™] 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
- 2. Add EasySep® Mouse Hematopoietic Progenitor Cell Enrichment Cocktail at 50 µL/mL cells (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for 15 minutes
- 3. Wash cells and resuspend at 1 x 108 cells/mL in recommended medium.
 - Note: The wash step is recommended if a high depletion of lineage antigen positive cells is required and the start number of desired cells is low. However, if high recovery is more desirable than high purity, it is recommended to leave out the wash step.
- 4. Add EasySep® Biotin Selection Cocktail at 100 μL/mL cells (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate at 2 - 8°C for 15 minutes.
- 5. Vortex EasySep® Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds to ensure that the particles are in a uniform suspension with no visible aggregates
- 6. Add the magnetic particles at 50 μ L/mL cells (e.g. for 2 mL of cells add 100 μ L of particles). Mix well and incubate at 2 - 8°C for 10 minutes.
 - Note: For increased depletion of lineage antigen positive cells, magnetic particles may be added at 75 uL/mL of cells. This will increase purity but will decrease recovery of cells
- 7. Bring the cell suspension to a total volume of 5 mL (for < 4 x 108 cells) or 10 mL (for 4 - 8.5 x 108 cells) by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for 3 minutes.
- 8. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 mL tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep® Magnet, Leave the magnet and tube inverted for 2 - 3 seconds, then return to upright position. Do not shake or blot off any drops that may remain hanging from the mouth of the tube. The enriched cells are now ready for use.

Normal Rat Serum

0.5 mL 1.0 mL 1.0 mL



+EasySep® *

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000), or "The Big Easy" (Catalog #18001), or RoboSep® (Catalog #20000). EasySep® Magnet

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Mouse Hematopoietic Progenitor Enrichment Cocktail, EasySep® Biotin Selection Cocktail and EasySep® Mouse Progenitor Magnetic Microparticles label lineage antigen (CD5, CD11b, CD19, CD45R, Ly-6G/C, TER119, 7-4) positive cells for magnetic separation. These reagents are designed to enrich hematopoietic stem cells and progenitor cells from mouse bone marrow cell suspensions by depletion of lineage positive cells.

EASYSEP® LABELING OF MOUSE CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using biotinylated antibodies against cell surface antigens expressed on the unwanted cells, and bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and biotin (Figure 1). Magnetically labeled cells are then separated from unlabeled target cells using the EasySep® procedure (reverse side).

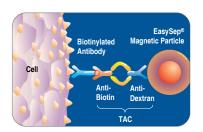


Figure 1. Schematic Drawing of EasySep® TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

BONE MARROW. Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23-gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes, discard supernatant and resuspend cells at 1 x 108 cells/mL in recommended medium. Add 5% rat serum (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

OPTIMAL CELL NUMBER. We do not recommend the use of fewer than 5 x 10⁷ cells per separation as this may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is RoboSep® Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free. Hank's Balanced Salt Solution can be used in place of PBS.

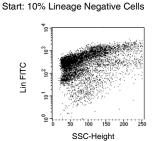
ASSESSING PURITY. Mouse hematopoietic stem cells (HSCs) and closely related primitive progenitors are distinguished from the majority of bone marrow cells by their lack of expression of markers specific for maturing blood cells (lineage antigens, e.g. CD3, CD11b, CD19, CD45R, GR1, and TER119). In many mouse strains, HSCs and primitive progenitors are positive for SCA1 (Ly-6A/E) and cKIT (Lin-SCA1+KIT+ phenotype). 1,2 More mature erythroid, myeloid and megakaryocyte progenitor cells are also Lin and cKIT+, but negative for SCA1 (Lin SCA1-KIT* phenotype). Mouse HSCs and progenitors are heterogeneous for other antigens, e.g., CD34 and THY1. The purity of these subsets after progenitor enrichment can be assessed by flow cytometry after staining with a cocktail of fluorescently-labeled antibodies against lineage antigens, cKIT, SCA1, CD34 and/or THY1.1. The recommended antibody clones for lineage antigen staining are 145-2C11 (CD3), RA3-6B2 (CD45R/B220, Catalog #10711), RB6-8C5 (GR1), Catalog #10717), M1/70 (MAC1/CD11b, Catalog #10705), 1D3 (CD19, Catalog #10707), TER-119 (TER119/Ly-76, Catalog #10729).

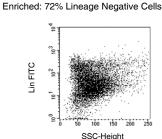
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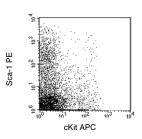
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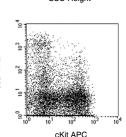
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TYPICAL EASYSEP® MOUSE HEMATOPOIETIC CELL ENRICHMENT PROFILE:









Starting with a mouse bone marrow cell suspension, the lineage antigen negative cell content of the enriched fraction typically ranges from 60-90%

COMPONENT DESCRIPTIONS:

EASYSEP® MOUSE HEMATOPOIETIC CELL ENRICHMENT COCKTAIL

CODE #19756C.1

This cocktail contains a combination of biotinylated monoclonal antibodies purified from rat ascites fluid or hybridoma culture supernatant. The monoclonal antibodies are purified by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are directed against cell surface antigens on mouse cells of hematopoietic origin (CD5, CD11b, CD19, CD45R, Ly-6G/C(GR1), TER119, 7-4). This cocktail is supplied in Phosphate Buffered Saline. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EASYSEP® BIOTIN SELECTION COCKTAIL

CODE #19153

This cocktail is a combination of two mouse IgG₁ monoclonal antibodies against biotin and dextran purified from hybridoma culture supernatant. These antibodies are bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse IgG₁. This cocktail is supplied in PBS. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EASYSEP® MOUSE PROGENITOR (M PROG) **MAGNETIC MICROPARTICLES**

CODE #19350

A suspension of magnetic dextran iron particles in TRIS buffer.

NORMAL BAT SERUM

CODE #13551

This normal rat serum is used to prevent non-specific binding of rat antibodies to mouse cells. It has been certified by the manufacturer to be mycoplasma-free.

STABILITY AND STORAGE:

EASYSEP® MOUSE HEMATOPOEITIC CELL ENRICHMENT COCKTAIL EASYSEP® BIOTIN SELECTION COCKTAIL EASYSEP® MOUSE PROGENITOR (M PROG) MAGNETIC MICROPARTICLES

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze this product. This product may be shipped at room temperature (15 -25°C), and should be refrigerated upon receipt.

NORMAL BAT SERUM

Product stable at 2 - 8°C until expiry date as indicated on label. Stable for at least 2 years when stored at -20°C. Contents have been sterility tested.

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