PlusCellect™

Mouse Sca-1^{+} Stem Cells Kit

Catalog Number PLS1226

For the isolation of stem/progenitor cells expressing Sca-1 via a positive selection principle.

This kit contains sufficient reagents for 20 tests (up to 1 x 10^{9} total cells).

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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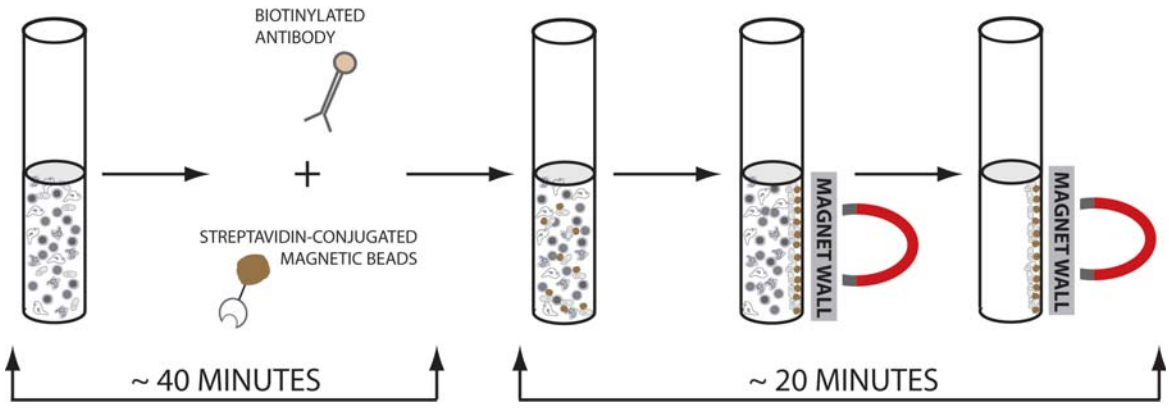
INTRODUCTION

Sca-1 is a membrane-anchored protein from the murine Ly-6 family. Its expression on multipotent hematopoietic stem cells (HSC) has been extensively used to enrich for HSCs (1 - 2). Essentially all HSCs express Sca-1 in *Ly6.2* strains, such as *C57/BL6*, although only 25% of HSCs express Sca-1 in *Ly6.1* strains, such as *BALB/c* (3). Sca-1 is required for the regulation of hematopoietic stem cell self-renewal and the development of specific progenitor populations (4). Outside the hematopoietic system, Sca-1 is expressed by stem cells, progenitor cells, and differentiated cell types in a wide variety of tissues and organs, and it has recently been identified as a marker for tumor-initiating cells (1, 5 - 7). Immunomagnetic cell isolation with Sca-1 can be used in combination with other markers for enrichment of stem, progenitor, and cancer-initiating cells (1, 7).

PRINCIPLE OF THE TEST

Cell isolation is done by positive selection in a test tube by tagging the cells of interest with a biotinylated antibody followed by the addition of Streptavidin-conjugated magnetic particles (MagCellect™ Streptavidin Ferrofluid or equivalent). The tube with the cell suspension is then placed in a magnet. Magnetically tagged cells will migrate toward the tube wall on the magnet side (desired cell population), leaving the untagged (unwanted) cells in suspension. Unwanted cells are first removed by aspiration while the tube remains in the magnet. The tube containing the magnetically selected (desired) cells is then removed from the magnet, and the cells are resuspended in PlusCellect Buffer or tissue culture media. To detect the presence of positively-selected cells or to assess the efficiency of enrichment, selected cells may be stained with the PE-conjugated antibody provided.



PlusCellect kits work with any single-cell suspension preparation. Cell suspensions can be prepared and stained by traditional methods or by following the instructions outlined on pages 4 and 6, respectively.

INTENDED USE

The Mouse Sca-1$^+$ PlusCellect Kit is designed to directly isolate stem/progenitor cells from mouse bone marrow via a positive selection principle. The resulting cell preparation is highly enriched for Sca-1$^+$ cells. Purity of recovered Sca-1$^+$ cells typically ranges between 65 - 85%.

MATERIALS PROVIDED

Mouse Sca-1 Selection Antibody (Part 965636) - 625 µL of biotinylated rat anti-mouse Sca-1 antibody.

Mouse Sca-1 Detection Antibody (Part 965637) - 250 µL of PE-conjugated rat anti-mouse Sca-1 antibody.

10X PlusCellect Buffer (Part 895921) - 50 mL of a proprietary formulation.

STORAGE

Reagents are stable for 12 months from the date of receipt when stored **in the dark at 2 - 8° C. DO NOT FREEZE.**

OTHER MATERIALS REQUIRED

- MagCellect Streptavidin Ferrofluid* (R&D Systems, Catalog # MAG999 or equivalent)
- MagCellect Magnet* (R&D Systems, Catalog # MAG997 or equivalent)
- 12 x 75 mm (5 mL) or 17 x 100 mm (15 mL) polystyrene round bottom tubes (Falcon, Catalog # 352008, 352006, or equivalent)
- 15 mL conical centrifuge tubes (Corning Costar, Catalog # 3375 or equivalent)
- Sterile Pasteur pipettes or transfer pipettes (Fisher Scientific, Catalog # 13-711-9B or equivalent)
- Phosphate-Buffered Saline (PBS)
- Benchtop centrifuge
- 2 - 8° C refrigerator
- Deionized water

If using bone marrow cells, the following supplies are also required:

- Fetal Bovine Serum (FBS)
- Mouse Lyse Buffer (R&D Systems, Catalog # FC003 or equivalent)
- 70 µm cell strainer (Falcon, Catalog # 352350 or equivalent)

**While optimized for R&D Systems' reagents and supplies, the PlusCellect kit was tested in combination with EasySep™ (StemCell Technologies), iMag™ (Becton Dickinson), and Streptavidin Microbeads™ (Miltenyi Biotec) magnetic beads and magnets. When using other supplier's magnetic selection systems, the protocol may need to be adapted according to the supplier's directions for optimal performance.*

Please note that PlusCellect kits only work with streptavidin-based magnetic beads.

PRECAUTION

The PE-conjugated detection antibody provided in this kit contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

REAGENT PREPARATION

1X PlusCellect Buffer - Prepare 25 mL of 1X PlusCellect Buffer for each sample to be processed by mixing 2.5 mL of 10X PlusCellect Buffer with 22.5 mL of sterile deionized or distilled water. The 1X PlusCellect Buffer should be kept on ice or refrigerated.

CELL PREPARATION

PlusCellect kits work with any single-cell suspension preparation. Cell suspensions can be prepared by traditional methods or by following the instructions below. Use sterile technique if the cells are to be used for *in vitro* culture or *in vivo* assays after enrichment.

Preparation of Bone Marrow Cells

1. Harvest the hind leg bones of mice* and prepare a mononuclear suspension of bone marrow cells using traditional methods (8).
2. Centrifuge the cells for 5 minutes at 400 x g and 2 - 8° C. Remove the supernate and resuspend the cells in 10 mL of cold PBS with 2% FBS by gently pipetting to generate a single cell suspension. Filter the cell suspension through a 70 μm cell strainer (Falcon, Catalog # 352350 or equivalent) to remove cell clumps.
3. After filtration, the cells can be used directly for selection. Or, if desired, the red blood cells (RBC) can be removed. To remove RBC, resuspend the cells in 5 mL of Mouse Lyse Buffer followed by centrifugation for 5 minutes at 400 x g and 2 - 8° C. Remove the supernate and resuspend the cells in cold 1X PlusCellect Buffer. Count the viable cells. After RBC lysis, one mouse typically yields 30 - 60 x 10^6 cells.

**Sca-1 expression in bone marrow cells from different mouse strains is variable. While Sca-1 expression in C57/BL6 strains is abundant in bone marrow, it is only expressed in a small subset of cells from BALB/c mice (3).*

CELL SELECTION PROCEDURE

Cells and reagents should be kept at 2 - 8° C. Incubations should be performed in a 2 - 8° C refrigerator. Do not perform incubations in an ice bath. Excessively low temperatures can slow the kinetics of the optimized reactions.

Note: *This procedure describes the processing of less than1 x 10^7 total cells using 5 mL tubes. Please refer to the Technical Hints section for processing other cell numbers.*

1. Prepare a single-cell suspension by traditional methods or by following the instructions outlined in the Cell Preparation section. Cells must be suspended in cold 1X PlusCellect Buffer at a density of approximately 1 x 10^7 cells/mL prior to beginning the procedure.
2. Place 1 x 10^7 cells (1.0 mL) into a 15 mL conical centrifuge tube.

   Note: *If necessary, block Fc receptor sites by adding 100 μg of mouse IgG in a volume not exceeding 100 μL. Incubate for 10 minutes in a refrigerator at 2 - 8° C.*
3. Add 25 μL of Mouse Sca-1 Selection Antibody. Gently mix the cell/antibody suspension, avoiding bubble formation, and incubate for 15 minutes at 2 - 8° C in a refrigerator. At the end of the incubation period, wash the cell suspension by adding 9 mL of cold 1X PlusCellect Buffer and centrifuge at 300 x g for 8 minutes. **Completely** remove the supernate and resuspend the cell pellet by gently pipetting 1 mL of cold 1X PlusCellect Buffer into the tube.
4. Add 50 μL of MagCellect Streptavidin Ferrofluid magnetic beads (or equivalent) to the cell suspension. Mix gently and incubate for 15 minutes at 2 - 8° C in a refrigerator.

   Note: *If using a magnetic selection system other than MagCellect, this part of the procedure will need to be adapted according to the supplier's instructions.*
5. At the end of the incubation period, wash the cell suspension by adding 9 mL of cold 1X PlusCellect Buffer and centrifuge at 300 x g for 8 minutes. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 2 mL of cold 1X PlusCellect Buffer into the tube. Transfer the cell suspension to a 5 mL reaction tube.
6. Place the reaction tube in the MagCellect magnet (or equivalent) that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature (18 - 25° C). Magnetically tagged **(desired)** cells will migrate toward the magnet, leaving the untagged (unwanted) cells in suspension in the supernatant.
7. While the tube is still in the magnet, remove unwanted cells by carefully aspirating all of the reaction supernate with a sterile Pasteur pipette or transfer pipette. Discard the supernatant.
8. Remove the tube containing the magnetically selected cells from the magnet and resuspend cells by adding 2.0 mL of cold 1X PlusCellect Buffer.
9. To complete the cell isolation procedure, repeat steps 6 - 7 at least once more with the resuspended cell fraction.

   Note: *If purity of the cell selection is critical, repeat this step one or two more times.*
10. Remove the tube containing the magnetically selected cells from the magnet and resuspend the cells by adding 1 - 2 mL of 1X PlusCellect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated Sca-1^+ cells. The cells are now ready to be counted, stained, and used in other downstream applications.
11. If the isolated Sca-1^+ cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100 μL of 1X PlusCellect Buffer and stain them using 10 μL of Mouse Sca-1 Detection Antibody. Proceed as usual with standard staining procedures.

CELL STAINING PROCEDURE

After successfully selecting the desired cell population, cells can be stained by traditional methods or by following the instructions below.

1. Add 100 μL of the positively selected cells to a 5 mL tube.
2. Add 10 μL of Mouse Sca-1 Detection Antibody.
3. Incubate for 30 - 45 minutes at 2 - 8° C.
4. Following this incubation, remove the unreacted antibody by washing the cells twice in 2 mL of 1X PlusCellect Buffer or PBS.
5. Resuspend the cells in 200 - 400 μL of 1X PlusCellect Buffer or PBS for final flow cytometric analysis.

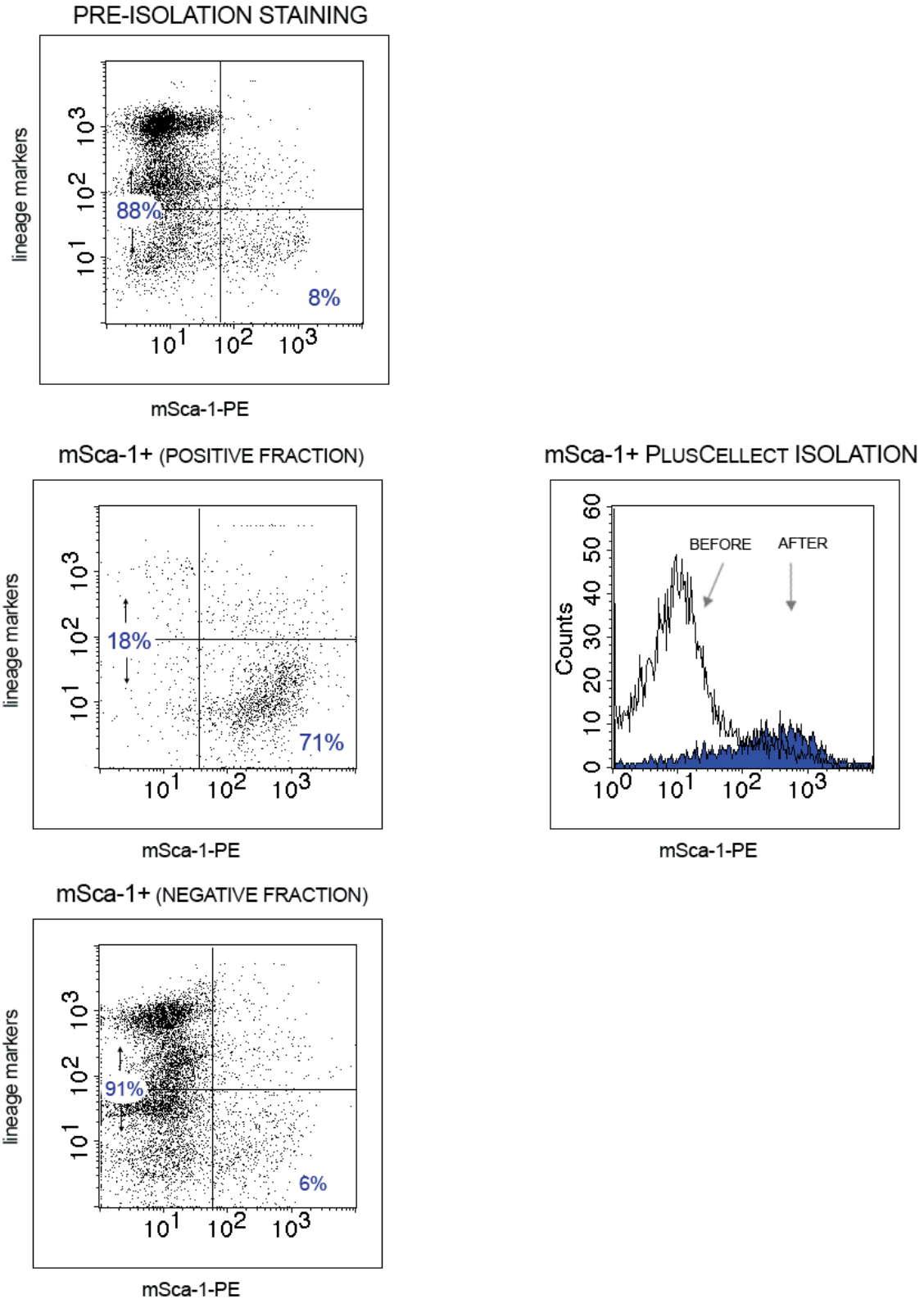
TECHNICAL HINTS

- If sterile cells are required following the cell selection, the entire procedure should be carried out in a laminar flow hood to maintain sterile conditions. Use sterile equipment when pipetting reagents that will be reused at a later date.
- Avoid antibody capping on cell surfaces and non-specific cell tagging by working quickly, by keeping cells and solutions cold through the use of pre-cooled solutions, and by adhering to the incubation times and temperatures specified in the procedure. Increased temperature and prolonged incubation times may lead to non-specific cell labeling, which may result in lowered cell purity and yield.
- When processing different numbers of cells, observe the following guidelines:
  - Keep the biotinylated antibody and ferrofluid incubation times the same.
  - Keep the cell density at 1 x 10^7 cells/mL.
  - If blocking, add 100 μg of mouse IgG per 10^7 cells being processed.
  - Add 5 μL of the biotinylated antibody per additional 10^7 cells being processed.
  - Add 10 μL of Streptavidin Ferrofluid per additional 10^7 cells being processed **to a maximum of 125 μL.**
- When processing 2 x 10^8 cells or fewer, use the 12 x 75 mm (5 mL) tubes with the MagCellect magnet horizontally positioned to accommodate up to six 5 mL tubes. **Do not process more than 2 x 10^8 cells in each 5 mL tube, and do not exceed a total reaction volume of 3 mL in each tube.** A reaction volume of 3 mL is recommended when processing 2 x 10^8 cells. A reaction volume of 1 mL is recommended when processing 5 x 10^7 or fewer cells. **Reaction volume adjustments must be made using 1X PlusCellect Buffer** just prior to the magnetic separation step.
- When processing greater than 2 x 10^8 cells, use 17 x 100 mm (15 mL) tubes with the MagCellect magnet vertically positioned to accommodate up to two 15 mL tubes. **Do not process more than 6 x 10^8 cells in each 15 mL tube, and do not exceed a total reaction volume of 9 mL in each tube.** When using this larger tube, increase the reaction volume before the magnetic separation step according to the following formula: 3 mL for each 2 x 10^8 cells processed. Increase the magnetic incubation time (step 6 of the Cell Selection Procedure) to 8 minutes. Reaction volume adjustments must be made using 1X PlusCellect Buffer just prior to the magnetic separation step.

TYPICAL DATA

Direct isolation of C57/BL6 mouse Sca-1$^+$ bone marrow cells using the PlusCellect Mouse Sca-1$^+$ Stem Cells kit. Samples were stained with Mouse Sca-1 PlusCellect Detection Antibody and with a mix of APC-conjugated lineage antibodies to assess separation efficiency.

PlusCellect Isolation of Mouse Sca-1+ Bone Marrow Cells



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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Possible Solution
Low yield of positively selected cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris are very sticky and will preferentially bind to the magnetic beads. If the cell preparation contains too many dead cells or debris, your magnetically selected fraction will be contaminated. **Make sure the cell preparation contains a minimal amount of dead cells or cell debris**. Test a small sample of cells with a vital dye before performing the cell selection procedure. The presence of cell debris is also easily identified in the FSS/SSC flow cytometry analysis.
	Cell aggregates	Cell aggregates will interfere with both cell selection and detection. **Make sure the cell preparation is a single-cell suspension before performing the cell selection procedure**. A small sample of cells can be tested with a vital dye before performing the cell selection procedure to ensure a healthy single-cell suspension.
	Few positive cell targets	If the cell fraction to be isolated contains less than ~50,000 cells, and it represents less than 1% of the total cell preparation, recovery could be affected. For a better yield, **increase the number of cells in your starting population**, if possible, or consider performing a pre-enrichment step by removing undesirable cells (R&D Systems has MagCellect or PlusCellect kits for negative selection of undesirable cells).
	Poor magnetic selection	When removing unwanted cells in step 7 of the Cell Selection Procedure, **make sure the tube in the magnet does not move.** If the tube is allowed to move or shift, positive cells that should be magnetically attached to the magnet might become loose. If the placement of the tube in the magnet is not tight, immobilize it with an adhesive tape. Also, be sure to aspirate the supernatant very carefully when removing the unwanted cells. Strong pipetting might release positive cells from the magnet.
Low purity of positively selected cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris are very sticky and will preferentially bind to the magnetic beads. If the cell preparation contains too many dead cells or debris, your magnetically selected fraction will be contaminated. **Make sure the cell preparation contains a minimal amount of dead cells or cell debris**. Test a small sample of cells with a vital dye before performing the cell selection procedure.
	Few positive cell targets	If the cell fraction to be isolated contains less than ~50,000 cells, and it represents less than 1% of the total cell preparation, purity of the isolated cells could be affected. For a better purity and yield, **increase the number of cells in the sample**, if possible, or consider performing a pre-enrichment step by removing undesirable cells (R&D Systems has MagCellect or PlusCellect kits for negative selection of undesirable cells).

Problem	Possible Cause	Possible Solution
Low purity of positively selected cells	Positive cells not washed well	**Extra washes** can be performed subjecting the cells to an extra step of magnetic migration (steps 7 and 8 of the Cell Selection Procedure). Additional magnetic selection steps could increase cell purity (typically ~5% increase) of the target population. Keep in mind that with every added step, a reduced yield can be expected.
No recovered cells	Insufficient positive cell targets	If the cell fraction to be isolated contains very few target cells or the positive cells represent a very small fraction of the total cell preparation, recovery could be significantly reduced and positive cells could be lost in the procedure. For a better yield, **increase the number of cells in the starting population** and/or **consider performing a pre-enrichment step** by removing undesirable cells (R&D Systems has MagCellect or PlusCellect kits for negative selection of undesirable cells).
	Cells lost in the process	If the cell fraction to be isolated contains very few target cells, there will be a low number of positively selected cells attached to the magnet, which may easily be lost during washes in steps 8 and 9 of the Cell Selection Procedure. If dealing with very few expected target cells, purity may need to be sacrificed for the sake of recovery. **Consider skipping steps 8 and 9 to avoid losing target cells.** Additionally, **make sure that cells are not lost during magnetic selection** in step 7 of the Cell Selection Procedure. Please refer to Poor Magnetic Selection on the first page of the Troubleshooting Guide for more details.
Biologically inactive recovered cells	The antibody or magnetic bead surface could interfere with biological processes	If the attached antibody/magnetic bead complex on the cell surface could be a problem, **culture the cells in the appropriate media** for a few hours or overnight to solve the issue. When in culture, and particularly while remaking the cell membrane during cell division, the antibody/magnetic bead complexes on the cell surface should be lost. Make sure the cells are cultured long enough to allow this process to occur.
Unusual staining of positive cells in flow cytometry	The antibody or magnetic bead surface could affect flow cytometric analysis	**No correction needed.** There are two primary reasons that this would occur. Magnetic beads on the cell surface could slightly change the cells' scattering characteristics, and the presence of the selection antibody/magnetic bead complex on the cell surface might slightly quench the fluorescence of the conjugated detection antibody. In both cases, the shift should be minor.

NOTES

EasySep™ is a trademark of StemCell Technologies
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