# MagCellect™ Mouse Mesenchymal Stem Cell Isolation Kit

**Catalog Number:** MAGM212B

## INTENDED USE

This MagCellect™ Mouse Mesenchymal Stem Cell Isolation Kit is designed to isolate mesenchymal stem cells via a negative selection principle. The resulting cell preparation is highly enriched with mesenchymal stem cells. The purity of recovered cells ranges between 75- 95%.

## PRINCIPLE OF SELECTION

A single-cell suspension is first incubated with the Mouse Mesenchymal Stem Cell Biotinylated Antibody Cocktail which targets the unwanted cells. Streptavidin Ferrofluid is added to the reaction which allows the streptavidin-coated nanoparticles to interact with the biotinylated antibody tagged cells. The tube containing the cell suspension is then placed within a magnetic field. Magnetically tagged cells will migrate toward the magnet (unwanted cell fraction), leaving the untagged cells or desired cell population in suspension to be harvested by aspiration while the tube remains in the magnetic field. The enriched cell preparation is then available for a variety of applications including tissue culture, immune status monitoring, and flow cytometry.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. **DO NOT FREEZE.**
This kit contains sufficient reagents to process 300 x $10^6$ total cells; up to 12 isolations.

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/DILUTED MATERIAL |
|---|---|---|---|
| **Mouse Mesenchymal Stem Cell Biotinylated Antibody Cocktail** | 860301 | 0.30 mL of a phosphate buffered solution containing BSA and preservative. | May be stored for up to 3 months at 2-8 ºC.* |
| **Streptavidin Ferrofluid** | 860165 | 3.0 mL of a solution containing BSA and preservatives. | |
| **Plus Buffer (10X)** | 860131 | 10 mL of a 10-fold concentrated buffer. | May be stored for up to 3 months at 2-8 ºC.*<br>Use diluted buffer within 24 hours. |

* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Sterile deionized or distilled water
- MagCellect™ Magnet (R&D Systems®, Catalog # MAG997) or equivalent*
- 12 x 75 mm (5.0 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B) or equivalent
- Hanks' Balanced Salt solution

## PRECAUTION

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

**This MagCellect™ Kit is compatible with Miltenyi MidiMACS™ and Stem Cell Technologies EasySep® magnets and columns.*



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Bio-Techne is a trading name for R&D Systems

## 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 efficiently, keeping cells and solutions cold through the use of pre-cooled solutions and by adhering to the incubation times and temperatures specified in the protocol. Increased temperature and prolonged incubation times may lead to non-specific cell labeling thus lowering cell purity and yield.
- Use the following table for recommended quantities to be used in steps 2-4 of the Cell Selection Procedure:

| Number of Cells in Starting Preparation | $25 \times 10^6$ | $50 \times 10^6$ |
|---|---|---|
| Reaction Volume | 0.5 mL | 0.5 mL |
| Mouse Mesenchymal Stem Cell Biotinylated Antibody Cocktail | 25 µL | 50 µL |
| Streptavidin Ferrofluid | 250 µL | 400 µL |

## CELL PREPARATION

Use preferred or traditional methods to prepare a single cell suspension from mouse bone marrow or compact bone.
For a general protocol to isolate compact bone and bone marrow cells from mice, refer to:

1. Soleimani, M. and S. Nadri (2009) Nature Protocols **4**(1):102.
2. Short, B.J. *et al.* (2009) Methods Mol. Biol. **482**:259.

**This kit has been successfully used to isolate mesenchymal stem cells from both bone marrow and compact bone.**

## PROTOCOL

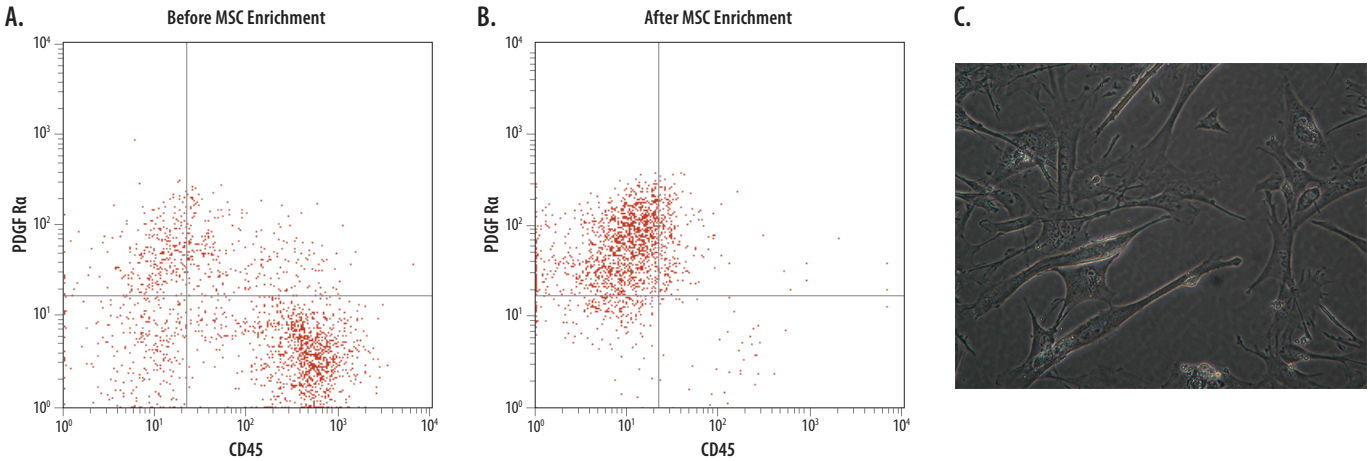
1. Generate a single cell suspension of compact bone or bone marrow cells in Hanks' Balanced Salt Solution (HBSS) (or other preferred media) supplemented with 10% bovine serum. To remove cell clumps and/or debris, pass the suspended cells through a 40-70 µm nylon cell strainer.
2. Wash the cells once with HBSS + 10% serum and spin down the cells for 10 minutes at 200 x g.
3. Decant the supernatant and disrupt the cell pellet by "racking" the tube. If necessary to remove red blood cells, resuspend the cells in R&D Systems® Mouse Erythrocyte Lysing Kit (Catalog # WL2000) that has been diluted to 1X strength with sterile deionized or distilled water and quickly vortex the tube. A reaction volume of 1.0 mL of 1X M Lyse solution is recommended.
4. Spin the cells for 10 minutes at 200 x g and resuspend the cells in a small volume of cold 1X Plus Buffer.
5. Perform a cell count and adjust the cell concentration to $50 \times 10^6$ cells/mL with cold 1X Plus Buffer.
6. Continue the cell selection by referring to step 1 of the Cell Selection Procedure.

## CELL SELECTION PROCEDURE

This procedure is for the processing of 25 x $10^6$ total cells using 5.0 mL tubes and the MagCellect™ Magnet. It is not recommended to use less than 10 x $10^6$ total cells. Cells and reagents should be kept cold using an ice bath or a refrigerator. **Reaction incubations must be carried out at 2-8 °C in a refrigerator and not in an ice bath to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Prepare 2.5 mL of 1X Plus Buffer for each 50 x $10^6$ cells to be processed by mixing 250 µL of Plus Buffer (10X) with 2.25 mL of sterile deionized or distilled water. The 1X Plus Buffer should be kept on ice or refrigerated and used within 24 hours.
2. Prepare a single cell suspension. Cells must be suspended in cold 1X Plus Buffer prior to beginning the procedure and be at a cell density of 50 x $10^6$ cells/mL.
3. Transfer 25 x $10^6$ cells (0.5 mL volume) into a 5.0 mL polystyrene tube. Add 25 µL of Mouse Mesenchymal Stem Cell Biotinylated Antibody Cocktail. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2-8 °C in a refrigerator for 15 minutes.
4. At the end of the incubation period, wash the cell suspension by adding 9.0 mL of cold 1X Plus Buffer and centrifuge at 300 x g for 8 minutes. Completely remove the supernatant and resuspend the cell pellet by gently pipetting 1.0 mL of cold 1X Plus Buffer into the tube.
5. Add 250 µL of Streptavidin Ferrofluid to the cell suspension, mix gently, and incubate at 2-8 °C in a refrigerator for 15 minutes.
6. At the end of the incubation period, bring the volume of the reaction in the tube to 2.0 mL by adding 1.35 mL of 1X Plus Buffer. Mix gently to ensure that all reactants in the tube are in suspension.
7. Place the reaction tube in the MagCellect™ Magnet, and incubate for 6 minutes at room temperature. Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the untouched, desired cells in suspension in the supernatant.
8. Recovery of the desired cells is achieved as follows: While the tube is firmly held in the magnet, using a sterile Pasteur pipette or transfer pipette, carefully aspirate all of the reaction supernatant and place it in a new 5.0 mL tube. Remove the tube containing the magnetically trapped cells from the magnet, and discard.
9. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 6 and 7) with the new tube containing the recovered cells. The supernatant obtained at the end of these steps is the final depleted cell fraction containing the desired enriched Mesenchymal Stem Cells. The cells are now ready for counting and further downstream applications.

## DATA EXAMPLES




**Enrichment of MSCS from C57/BL6 Mouse Compact Bone Using the MagCellect™ Mouse Mesenchymal Stem Cell Isolation Kit.** Cells before **(A)** and after **(B)** mesenchymal stem cell enrichment were stained with Alexa 488-conjugated Anti-Mouse PDGF Rα (R&D Systems®, Catalog # FAB1062G) and an Anti-Mouse CD45-APC Monoclonal Antibody (R&D Systems®, Catalog # FAB114A). **(C)** Morphology of isolated, unstained cells demonstrating a typical mesenchymal stem/stromal cell shape.

## TROUBLESHOOTING GUIDE

Most difficulties arise from the following areas:

**Quality of the Cell Preparation:** Dead cells and debris might interfere with isolation and detection, affecting both the purity and recovery of intended cells. Cell aggregation might also affect the enrichment performance.

**Few Expected Target Cells:** If less than 100,000 expected target cells are present, recovery and/or purity of the isolation is compromised.

| Issue | Possible Cause | Possible Solution |
|---|---|---|
| **Low yield of isolated cells** | Poor cell preparation, too many dead cells, or cell debris | Dead cells and cell debris will affect the isolation efficiency. Make sure your 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 the cell selection. Make sure you have 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 expected cell targets | If the cell fraction to be isolated contains less than ~250,000 cells or 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 prior removal of known undesirable cells in your preparation (R&D Systems® has MagCellect™ 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, 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 adhesive tape. Also, be sure to aspirate the supernatant very carefully when removing the unwanted cells. Strong pipetting might release undesired cells from the magnet. |
| **Low purity of isolated cells** | Poor cell preparation, too many dead cells, or cell debris | Dead cells and cell debris will affect the isolation efficiency. Make sure your cell preparation contains a minimal amount of dead cells or cell debris. Test a small sample of your cells with a vital dye before performing the cell selection procedure. The presence of cell debris could also be easily identified in the FSS/SSC flow cytometry analysis. |
| | Few positive cell targets | If the cell fraction to be isolated contains less than ~250,000 cells or 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 prior removal of known undesirable cells in your preparation (R&D Systems® has MagCellect™ kits for negative selection of undesirable cells). |
| | Enriched cells not washed well | Extra washes can be performed subjecting the cells to an extra step of magnetic migration (steps 7-8 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 cells recovered** | Insufficient cell targets | If the cell fraction to be isolated represents a very small fraction of the total cell preparation, recovery could be significantly reduced. For a better yield, increase the number of cells in your starting population and/or consider performing a pre-enrichment step by removing undesirable cells (R&D Systems® has MagCellect™ kits for negative selection of undesirable cells). |