

MESENCHYMAL CELL KIT

Reference	Size
MCK-50T	50 test

PRODUCT DESCRIPTION

Reagent provided: Cocktail of fluorochrome-conjugated murine monoclonal antibodies.

Tested application: flow cytometry.

Storage buffer: Antibodies are supplied in a stabilization buffer containing protein matrix and 0.09% sodium azide (NaN₃).

Capacity: 50 tests or up to 5 x 10⁷ total cells

Recommended usage:

The Immunostep Mesenchymal Cell Kit features a validated, pre-optimized panel of fluorochrome-conjugated monoclonal antibodies designed for the standardized identification and immunophenotypic characterization of human multipotent mesenchymal stromal cells (MSCs) by multicolor flow cytometry. The assay architecture leverages the minimal phenotypic criteria established by the International Society for Cellular Therapy (ISCT). This formulation allows for the simultaneous assessment of essential MSC-positive markers (≥95% expression) and the exclusion of contaminating hematopoietic and endothelial lineages (≤2% expression) within single-vial determinations, maximizing workflow efficiency and minimizing sample commitment.

Presentation: liquid

Components & Reagents Provided

The kit comprises an optimized configuration of fluorochrome-conjugated monoclonal antibodies, formulated into three distinct multicolor panels and supplemented with dedicated single-color compensation controls:

- **Phenotyping Cocktail A (Vial A):** Contains CD90 FITC, CD105 PE, CD29 APC, and a pre-matched exclusion cocktail [CD45, CD19, HLA-DR, CD14, CD34] conjugated to PerCP.
- **Phenotyping Cocktail B (Vial B):** Contains CD44 FITC, CD166 PE, CD73 APC, and a pre-matched

exclusion cocktail [CD45, CD19, HLA-DR, CD14, CD34] conjugated to PerCP.

- **Isotype Control Cocktail (Vial C):** Contains pre-mixed Mouse IgG₁ FITC, Mouse IgG₁ PE, Mouse IgG₁ PerCP, and Mouse IgG₁ APC to accurately define non-specific binding thresholds.
- **Compensation Aliquots:** Four individual single-antibody vials provided for optimal compensation alignment of fluorescence spillover:
 - CD90 FITC
 - CD166 PE
 - CD44 PerCP
 - CD29 APC

Reagent and instrument requirements:

- **Staining buffer:** Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA
- **Flow cytometry** equipped with a red and blue laser.

CLINICAL RELEVANCE

Mesenchymal stromal cells (MSCs) are adult, fibroblast-like multipotent cells characterized by their capacity to self-renew and differentiate into lineages of mesodermal origin, such as adipocytes, chondroblasts, and osteoblasts. To ensure reproducibility and standardization across clinical and research fields, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) established minimal criteria for defining human MSCs:

1. **Plastic-adherence** under standard culture conditions.
2. **Positive expression** (≥ 95%) of surface markers CD105, CD73, and CD90.
3. **Lack of expression** (≤ 2%) of hematopoietic and endothelial markers: CD45, CD34, CD14 or CD11b, CD19 or CD79α, and HLA-DR. This Immunostep MSC Kit provides a comprehensive, pre-matched multicolor panel designed to screen these positive and negative surface molecules

simultaneously, reducing pipetting errors and sample commitment

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.

tech@immunostep.com.

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed.

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS

- a) The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
- b) Avoid microbial contamination of the reagent.
- c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
- d) Never mouth pipette.
- e) In the case of contact with skin, wash in plenty of water.
- f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
- g) Do not use after the expiry date indicated on the vial.
- h) Deviations from the recommended procedure could invalidate the analysis results.
- i) FOR RESEARCH USE ONLY.

- j) For professional use only.
- k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE PREPARATION

The following protocol is optimized for cultured and dissociated human MSCs. Cells must be single-cell suspended prior to staining.

Cell Surface Staining Protocol:

1. Label three 12 x 75 mm polystyrene tubes as **A, B, and C**.
2. Transfer up to 1×10^6 cells into each tube.
3. Centrifuge at **300g for 10 minutes** (or 540g for 5 minutes, according to laboratory standard). Gently aspirate the supernatant, leaving approximately 50 μ L of residual fluid.
4. Resuspend the cell pellet in each tube by adding 50 μ L of Staining Buffer.
5. Add **20 μ L of Vial A** into tube A, **20 μ L of Vial B** into tube B, and **20 μ L of Vial C** into tube C. Mix gently using a vortex mixer.
6. Incubate in the dark for **15 minutes at room temperature (20–25 °C)** or **30 minutes at 4 °C**.
7. Wash cells by adding 2 mL of Staining Buffer and centrifuge at the same validated settings.
8. Carefully aspirate the supernatant without disturbing the pellet, leaving ~50 μ L of fluid.
9. Resuspend the final cell pellet in **200–400 μ L of Staining Buffer**.
10. Analyze on the flow cytometer immediately. If analysis is delayed, store at 2–8 °C protected from light and acquire **within 3 hours post-staining**.

FLOW CYTOMETRY ANALYSIS

- Before sample acquisition, ensure the flow cytometer is properly calibrated, laser-aligned, and daily QC parameters are validated.
- Ensure that color compensation settings have been accurately established using the internal

compensation components included in this kit.

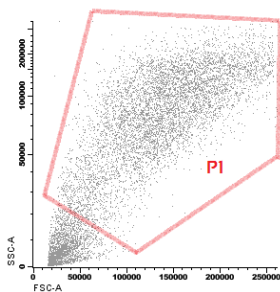
- Set light scatter parameters (FSC-A vs. SSC-A) on linear scale, and fluorescence channels (FITC, PE, PerCP, APC) on logarithmic scale.
- Set an appropriate FSC threshold/discriminator to exclude cellular debris, ensuring that the target MSC population is fully unmasked (Region PI).

Before acquiring samples, set to the minimum the Threshold or Discriminator in parameter FSC to minimize debris and ensure population of interest are included.

Gently mix the samples manually immediately prior to running on the flow cytometer to ensure thorough resuspension of cells and microspheres.

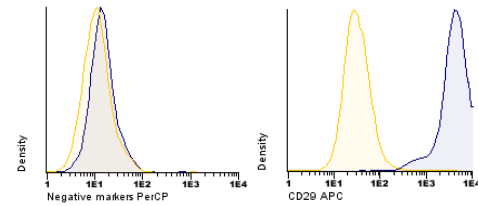
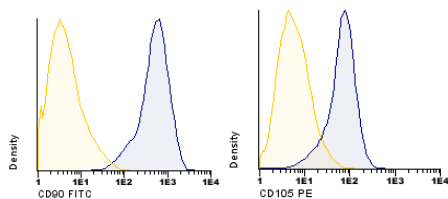
Acquire and store all events possible. It is recommended to acquire at a low or medium speed to avoid cell aggregates.

Set on the cytometer to store only the events in the region PI as imagen below:

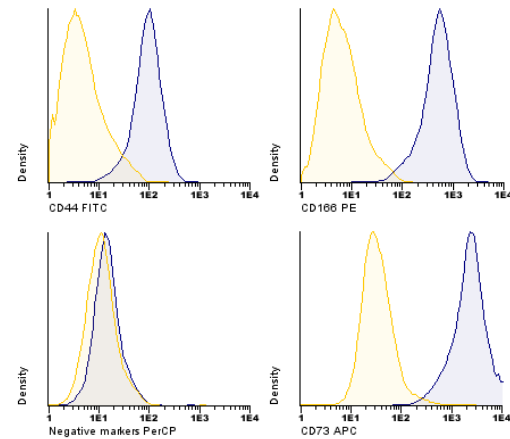


The following histograms correspond to the analysis of antigen expression in an expansion of bone marrow-derived MSCs (passage 3).

VIAL A + C



VIAL B + C



Annex 1: Compensation of flow cytometer

1. Label five 12 x 75 mm tubes as: **Blank, FITC, PE, PerCP, and APC.**
2. Add 0.5×10^6 cells into each tube. Centrifuge at 540g for 5 minutes, aspirate supernatant, and leave ~50 μ L of residual volume.
3. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 μ L of fluid. Add 50 μ L of buffer and mix gently with a vortex mixer.
4. Distribute the compensation reagents as follows:
 - **FITC tube:** Add 20 μ L of CD90 FITC.
 - **PE tube:** Add 20 μ L of CD166 PE.
 - **PerCP tube:** Add 20 μ L of CD44 PerCP.
 - **APC tube:** Add 20 μ L of CD29 APC.
 - **Blank tube:** Do not add any antibody.
5. Incubate in the dark for 15 minutes at room temperature (20–25 °C).
6. Wash by adding 2 mL of Staining Buffer, centrifuge at 540g for 5 minutes, and discard supernatant.
7. Resuspend each single-color pellet in 300 μ L of Staining Buffer and execute the compensation matrix algorithm on your cytometer software.

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

REFERENCES

1. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* 2008 Apr 10;2(4):313-9.
2. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* Oct 03;13(4):392-402.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8(4):315-7.

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