Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. / Fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com

# MESENCHYMAL CELL KIT

| Reference | Size |
|---|---|
| MCK-50T | 50 test |

## PRODUCT DESCRIPTION

**Reagent provided:** murine monoclonal antibodies combination.
**Tested application:** flow cytometry
**Storage buffer:** aqueous buffered solution containing protein stabilizer and 0.09% sodium azide ($NaN_3$).
**Recommended usage:** Immunostep's mesenchymal cell Kit, is intended for identification of human multipotent mesenchymal stromal cells by flow cytometry. This reagent is effective for direct immunofluorescence staining for flow cytometric analysis using ⩽1 µg/$10^6$ cells.
**Presentation:** liquid
**Reagentsrequired:** The kit is composed by a combination of differents murine monoclonal antibodies into 3 vials, A, B and C.

- Vial A: CD90 FITC, CD105 PE, CD45 PerCP, CD19 PerCP, HLA-DR PerCP, CD14 PerCP, CD34 PerCP, CD29 APC, 50 test (20 ul/test - 1 ml).
- Vial B: CD44 FITC, CD166 PE, CD45 PerCP, CD19 PerCP, HLA-DR PerCP, CD14 PerCP, CD34 PerCP, CD73 APC, 50 test (20 ul/test - 1 ml).
- Vial C: Isotype control IgG1 FITC, Isotype control IgG1 PE, Isotype control IgG1 PerCP, Isotype control IgG1, 50 test. (20 ul/test - 1 ml).

In addition the kit includes four aliquots of antibody to perform the compensation: CD90 FITC, CD166 PE, CD44 PerCP and CD29 APC (5 ul/test - 25 ul each one).

## CLINICAL RELEVANCE

Mesenchymal stromal cells (MSCs) are adult, fibroblast-like multipotent cells characterized by the ability to differentiate into tissues of mesodermal origin, such as adipocytes, chondroblasts, and osteoblasts[1]. First identified and isolated from the bone marrow (BM), MSCs can now be expanded from a variety of other tissues including adipose tissue (AT), umbilical cord blood (UCB), skin, tendon, muscle, and dental pulp2. MSCs can be isolated based on their ability to adhere to plastic culture dishes, and they are capable of significant expansion by consecutive in vitro passaging[(1)].

Some early research suggested that MSCs might also differentiate into many different types of cells that do not belong to the skeletal tissues, such as nerve cells, heart muscle cells, liver cells and endothelial cells, which form the inner layer of blood vessels. These results have not been confirmed to date. In some cases, it appears that the MSCs fused together with existing specialized cells, leading to false conclusions about the ability of MSCs to produce certain cell types. In other cases, the results were an artificial effect caused by chemicals used to grow the cells in the lab.

The considerable therapeutic potential of human multipotent mesenchymal stromal cells (MSC) has generated markedly increasing interest in a wide variety of biomedical disciplines. However, investigators report studies of MSC using different methods of isolation and expansion, and different approaches to characterizing the cells. Thus it is increasingly difficult to compare and contrast study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules.
Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. While these criteria will probably require modification as new knowledge unfolds, we believe this minimal set of standard criteria will foster a more uniform characterization of MSC and facilitate the exchange of data among investigators.

The MSC kit is based on the phenotypic signature described by The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT)[(2, 3)].

## 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.

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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 protocol is for working with cultured human MSCs.
Cell surface Protocol:

1. Transfer up 1x106 cells to a 12 x 75 mm polystyrene test each of the three tubes A, B and C.

2. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid. Add 50 ul of buffer and mix gently with a vortex mixer.

3. Add 20 µL of vial A into tube A, 20 ul of vial B into tube B and 20 ul of vial C into tube C. Mix gently with a vortex mixer. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory.

4. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.

5. Add 2 ml of buffer and mix gently with a vortex mixer.

6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.

7. Resuspend pellet adding 50 µL of buffer.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.

## FLOW CYTOMETRY ANALYSIS

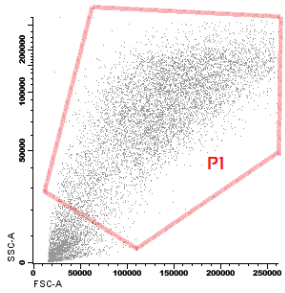
Before acquiring samples, verify the cytometer is correctly aligned and standardized for light scatter (FSC and SSC parameters must be set on linear amplification) and fluorescence intensity (FL1, FL2, FL3 and FL4) parameters must be set on logarithmic amplification) and colour compensation has been set following the instructions of the cytometer manufacturer.

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.

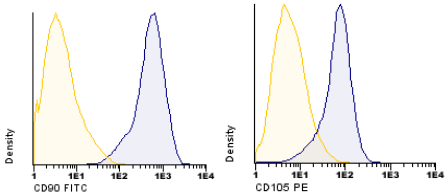
Acquire and store all events possible. It is recommended to acquire at a low or medium speed to avoid cell aggregates.

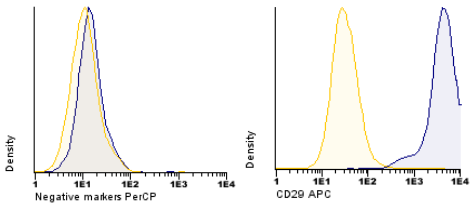
Set on the cytometer to store only the events in the region P1 as imagen below:



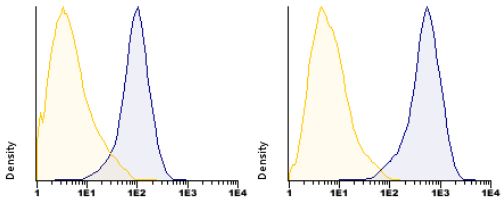

The following histograms correspond to the analysis of antigen expression in an expansion of bone marrow-derived MSCs (passage 3).

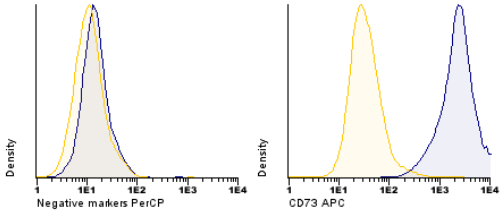
VIAL A + C




VIAL B + C



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**Annex 1: Compensation of flow cytometer**

1. Take five tubes each with 0,5 x106 cells to a 12 x 75 mm polystyrene test each of the five tubes Blank tube, FITC tube, PE tube, PerCP tube and APC tube.
2. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid. Add 50 ul of buffer and mix gently with a vortex mixer.
3. Add 5 µL of CD90 FITC into FITC tube, 5 ul of CD166 PE into PE, 5 ul of CD44 PerCP and 5 ul of CD29 APC.
4. Incubate in the dark at room temperature (20-25 ° C) for 15 minutes or at 4 °C for 30 minutes.
5. Add 2 ml of buffer and mix gently with a vortex mixer.
6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.
7. Resuspend pellet adding 200 µL of buffer in each tube.
8. Compensate instrument according to the flow cytometer manufacturer instructions.

## 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 Cell2008 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. Cytotherapy2006;8(4):315-7.

## MANUFACTURED BY

**Immunostep S.L**
Avda. Universidad de Coimbra, s/n
Cancer Research Center (CIC)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. (+34) 923 294 827
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