

BD Stemflow™ hMSC Analysis Kit

For reproducible multicolor phenotyping of MSC expansions

Features

ISCT-recommended panel for multicolor analysis of human multipotent mesenchymal stromal cells by flow cytometry

Delivers a streamlined solution for consistent, reproducible experiments with pre-titrated fluorochrome-conjugated antibody cocktails, isotype control cocktails, and compensation controls

Modular design for simultaneous analysis of additional positive or negative markers

Verified for the evaluation of bone marrow-derived hMSCs expanded in common MSC culture conditions

The BD Stemflow™ hMSC Analysis Kit provides a comprehensive system for the reliable, in-depth characterization of human bone marrow-derived multipotent mesenchymal stromal cells (BM-MSCs) based on the phenotypic signature described by The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT).¹

The ISCT has proposed a minimal set of three standard criteria to be used as the uniform definition of multipotent MSCs: 1) adherence to plastic, 2) specific surface antigen expression, and 3) multipotent differentiation potential. The phenotype of multipotent MSCs is defined to be, at a minimum, the cell surface co-expression of the antigens CD105, CD73, and CD90 ($\geq 95\%$ positive) and the absence of hematopoietic lineage markers CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA-DR ($\leq 2\%$ positive). The ISCT has emphasized that the optimal flow cytometric assay uses multicolor analysis to demonstrate that individual cells co-express unique MSC markers and lack hematopoietic antigen expression.¹

Multicolor Flow Cytometry for In-depth Analysis

Capitalizing on the powerful capabilities of multicolor flow cytometry, the kit allows researchers to perform multiparameter analysis at the single-cell level. Precise data on the percentage of cells positively expressing the defined cell surface markers of MSCs or contaminating hematopoietic cells can be obtained from samples of near-pure populations or even highly heterogeneous cell populations.

A Total Solution System to Minimize Variability

The BD Stemflow hMSC Analysis Kit is a multicolor flow cytometry assay containing pre-conjugated and pre-titrated cocktails of the ISCT-defined positive expression markers (CD105 PerCP-Cy™5.5/CD73 APC/CD90 FITC) and negative expression markers (CD45/CD34/CD11b/CD19/HLA-DR PE). The kit also contains the corresponding isotype controls to improve productivity and reduce assay-to-assay variability. For the optimization of MSC discrimination, single-stained cellular compensation controls are included to standardize instrument setup procedures and reduce day-to-day and lab-to-lab variability.

Modular Design to Accommodate Specific Needs

The ISCT encourages investigators to test for as many additional surface markers (both positive and negative) as may be necessary to demonstrate higher levels of purity for certain experiments. For immunophenotypic analyses beyond the minimal requirements, the modular architecture of the BD Stemflow hMSC Analysis Kit enables the easy addition of supplementary monoclonal antibodies against critical cell surface markers. A sample drop-in marker (CD44) and experimental protocol for expanded panel design are included in the kit.

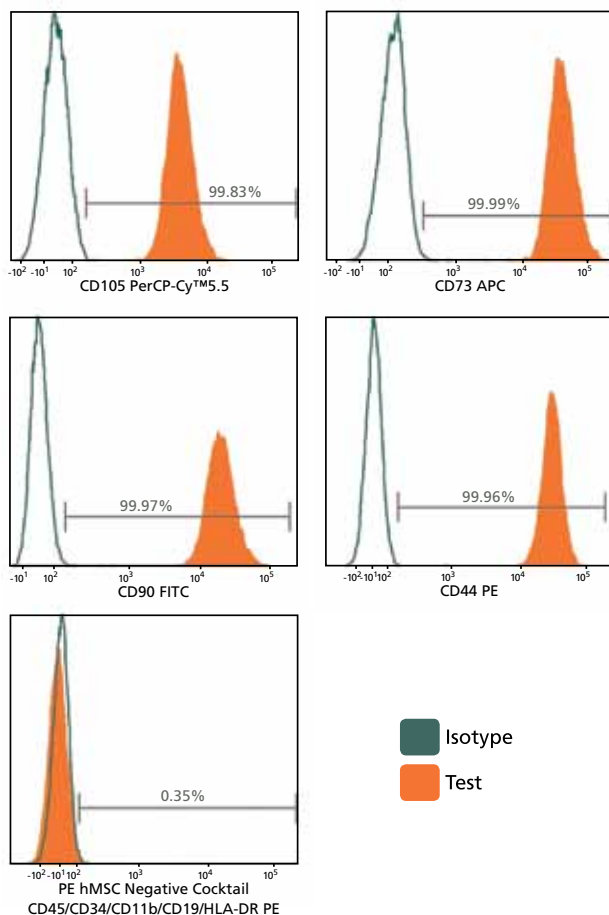


Figure 1. Analysis of positive and negative cell surface antigen expression in an expansion of bone marrow-derived MSCs (passage 3). Cells were detached using Accutase™ Cell Detachment Solution and analyzed with the BD Stemflow hMSC Analysis Kit. Cells were analyzed using a BD FACSCanto™ II flow cytometry system. The results indicate that the sample contains near-pure populations of hMSCs as defined by the minimal criteria for MSC surface antigen phenotyping.

Visit bdbiosciences.com/stemcell for more information.



BD Stemflow™ hMSC Analysis Kit

BD Biosciences has developed a large portfolio of research markers for the extensive analysis of MSCs. To download a complete list of purified and fluorochrome-conjugated positive and negative MSC research markers, please visit: bdbiosciences.com/stemcell.

Enabling the MSC Expansion and Analysis Workflow

This set of criteria proposed by the ISCT provides a uniform definition of human MSC isolation using a variety of methods for expansion under various conditions. The BD Stemflow hMSC Analysis Kit is a proven tool for rapidly evaluating the purity of BM-MSC cultures, between cell culture expansion passages, prior to storage, and before use in their final research applications (eg, in vitro differentiation).

Ordering Information

Description	Size	Cat. No.
BD Stemflow hMSC Analysis Kit	50 tests (1 x 10 ⁶ cells/test)	562245

BD Stemflow hMSC Analysis Kit contents

Positive Marker Cocktail
CD105 PerCP-Cy5.5/CD73 APC/CD90 FITC
Additional Positive Drop-In Marker
CD44 PE
Negative Marker Cocktail
CD45/CD34/CD11b/CD19/HLA-DR PE
Isotype Controls
mIgG ₁ , κ PerCP-Cy5.5/ mIgG ₁ , κ APC/ mIgG ₁ , κ FITC (for positive cocktail)
mIgG ₁ , κ/mIgG _{2a} , κ PE (for negative cocktail)
mIgG _{2b} , κ PE (for CD44 drop in)
Compensation Controls
CD105 PerCP-Cy5.5
CD73 APC
CD90 FITC
CD44 PE

Related Products

Description	Size	Cat. No.
BD Stemflow™ Human MSC Lineage Antibody Cocktail with Isotype Control Includes: PE hMSC Negative Cocktail (CD34, CD11b, CD19, CD45, HLA-DR) PE Isotype Control Cocktail (Mouse IgG ₁ , κ; Mouse IgG _{2a} , κ)	50 tests	562530
Accutase™ Cell Detachment Solution	100 mL	561527

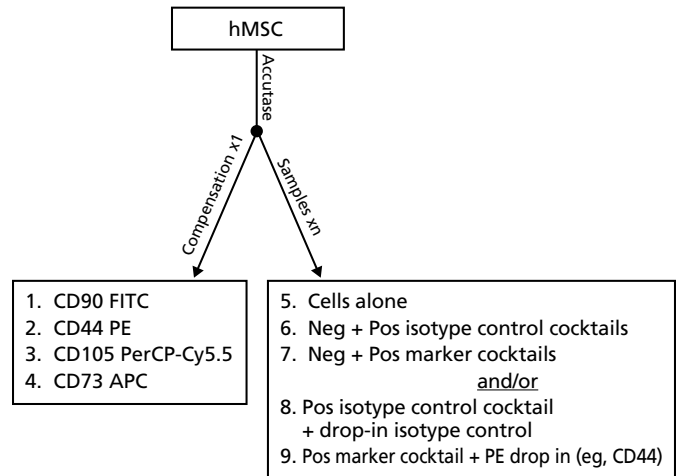


Figure 2. Workflow Schematic of the BD Stemflow hMSC Analysis Kit.

Human MSCs are dissociated with Accutase Cell Detachment Solution. Cells from one sample are then used to stain compensation controls (tubes 1 to 4). Cells from each sample to be analyzed are stained in a three- to five-tube experiment as diagrammed above. Cells from each sample are either not stained (tube 5), stained with the positive and negative isotype control cocktails (tube 6), or stained with positive and negative cocktails (tube 7). To expand the defined panel to test for the expression of additional markers, run the positive cocktail with the optional “drop-in” marker in the PE channel (tube 9) and its corresponding isotype control (tube 8). CD44 PE and the corresponding pre-conjugated isotype control are included in the kit. Additional colors of purified and fluorochrome-conjugated antibodies and isotype controls for MSC analysis are available from BD Biosciences.

References

1. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315-317.

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