Technical Data Sheet

Human MSC Analysis Kit

Product Information

Material Number: **562245**
Size: 50 Tests
Reactivity: Tested in Development: Human

Description

Human multipotent mesenchymal stromal cells (MSCs), also referred to as mesenchymal stem cells, are a rare population of adult stem cells that can be isolated from a variety of tissues. MSCs that have been isolated from bone marrow and subsequently cultured can differentiate to a variety of cell types, most notably adipocytes, osteocytes and chondrocytes. MSCs also have immunomodulatory effects in vivo and in vitro. In 2006, the International Society for Cellular Therapy (ISCT) proposed a cell surface marker panel for the minimal identification of human MSCs derived from bone marrow. Under this recommendation MSCs should be positive for CD73, CD90, and CD105, but be negative for CD34, CD45, CD11b or CD14, CD19 or CD79α, and HLA-DR. MSCs are also known to express numerous cell surface markers such as CD44, CD29, CD200, CD166, CD146 and CD271. In this kit we include the panel that was proposed by the ISCT. In addition, we designed this kit to be modular so that additional markers could be "dropped-in". Specifically, the MSC positive cocktail (FITC CD90, PerCP-Cy™5.5 CD105 and APC CD73) leaves the PE channel open to use in combination with the supplied negative MSC cocktail (PE CD45, PE CD34, PE CD11b, PE CD19 and PE HLA-DR), the included PE CD44 antibody conjugate, or a variety of other commercially available PE antibody conjugates. Multi color analysis minimizes cell numbers required for an assay and antibody cocktails facilitate a streamlined staining protocol to analyze multiple samples. Individual positive markers are included for compensation set-up.

Kit Components

Component	Description	Size	Vol. Per Test	Storage Buffer
51-9007661	PE hMSC Negative Cocktail	50 Test	20 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007662	PE hMSC Isotype Control Negative Cocktail	50 Test	20 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007663	hMSC Positive Cocktail	50 Test	20 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007664	hMSC Isotype Control Positive Cocktail	50 Test	20 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007657	FITC Mouse Anti-Human CD90	50 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007656	PE Mouse Anti-Human CD44	100 Test	5 µl	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide
51-9007655	PE Mouse IgG2b, k Isotype Control	50 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007649	APC Mouse Anti-Human CD73	50 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007648	PerCP-Cy™5.5 Mouse Anti-Human CD105 (Endoglin)	50 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide

Description of Kit Components

Vial	Contents	Purpose
hMSC Positive Cocktail	CD90 FITC (Clone: 5E10) CD105 PerCP-Cy5.5 (Clone: 266) CD73 APC (Clone: AD2)	Cocktail to positively identify hMSCs
hMSC Positive Isotype Control Cocktail	mIgG1, κ FITC (Clone: X40) mIgG1, κ PerCP-Cy5.5 (Clone: X40) mIgG1, κ APC (Clone: X40)	Corresponding Isotype Control for hMSC Positive Cocktail

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PE hMSC Negative Cocktail	CD34 PE (Clone:581) CD11b PE (Clone: ICRF44) CD19 PE (Clone: HIB19) CD45 PE (Clone: HI30) HLA-DR PE (Clone: G46-6)	Cocktail to identify potential contaminants
PE hMSC Negative Isotype Control Cocktail	mIgG1, κ PE (Clone: X40) mIgG2a, κ PE (Clone:G155-178)	Corresponding isotype control for PE hMSC Negative Cocktail
FITC Mouse Anti-human CD90	CD90 FITC (Clone: 5E10)	Compensation control
PE Mouse Anti-Human CD44	CD44 PE (Clone: G44-26)	Compensation control/MSC positive drop-in
PerCP-Cy™5.5 Mouse Anti-Human CD105	CD105 PerCP-Cy™5.5 (Clone: 266)	Compensation control
APC Mouse Anti-Human CD73	CD73 APC (Clone:AD2)	Compensation control
PE Mouse IgG2b, κ Isotype Control	mIgG2b, κ (Clone: 27-35)	Corresponding Isotype Control for PE Mouse Anti-Human CD44, when used as a drop in

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

Flow cytometry	Tested During Development

Recommended Assay Procedure:

(1) Detach cells using BD™ Accutase™ Cell Detachment Solution (Cat. No 561527) or similar detachment solution, wash cells and resuspend at a concentration of 1x10^7 cells/ml in BD Pharmingen™ Stain Buffer (Cat. No. 554656) or other appropriate staining buffer.
  (a) Cells can also be resuspended at a concentration of 5x10^6 cells/ml if cell number is a limiting factor.

(2) Label tubes and add antibodies as shown below:

Tube	Add (1 test size)
(1)	FITC Mouse Anti-Human CD90 (5µl)
(2)	PE Mouse Anti-Human CD44 (5µl)
(3)	PerCP-Cy™5.5 Mouse Anti-Human CD105 (5µl)
(4)	APC Mouse Anti-Human CD73 (5µl)
(5)	Nothing
(6)	hMSC Positive Isotype Control Cocktail (20µl) PE hMSC Negative Isotype Control Cocktail (20µl)
(7)	hMSC Positive Cocktail (20µl) PE hMSC Negative Cocktail (20µl) **And/or**
(8)	hMSC Positive Isotype Control Cocktail (20µl) Drop in isotype control (i.e. PE Mouse IgG2b, κ) (5 µl)
(9)	hMSC Positive Cocktail (20µl) PE Drop in (i.e. PE Mouse Anti-Human CD44) (5µl)

(3) Repeat tubes 5-7 and/or 8-9 for each additional cell sample.

(4) Add 100 µl of prepared cell suspension to tubes 1 through 9.

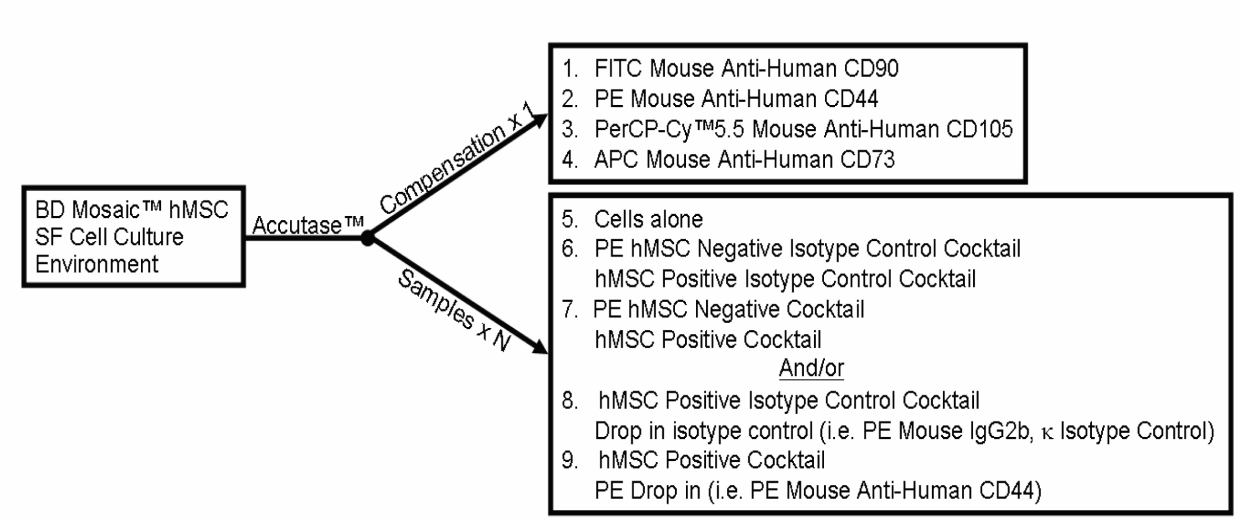
(5) Incubate tubes in the dark for 30 minutes (May be done on ice or at room temp)

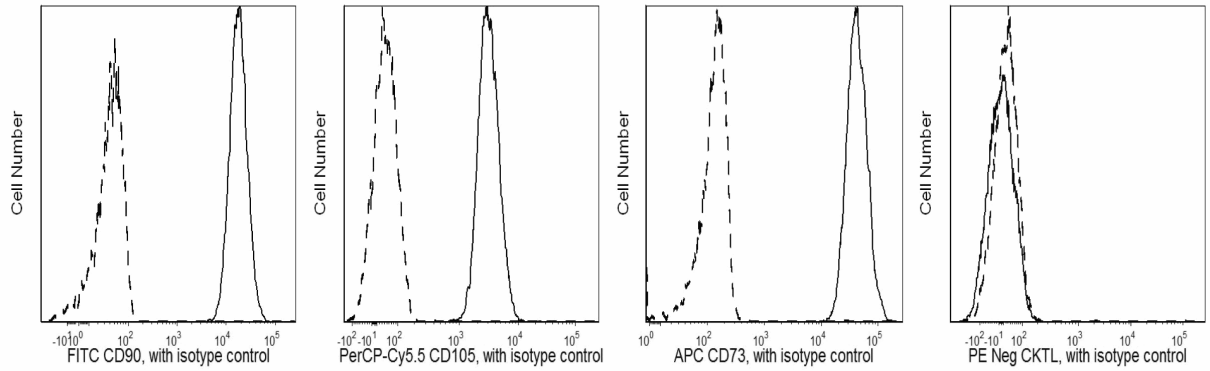
(6) Wash the cells twice with BD Pharmingen™ Stain Buffer (FBS) and resuspend at 300-500 µl in BD Pharmingen™ Stain Buffer (FBS) . Alternatively, 1X Washing/Staining Solution (1 x PBS,1% FCS, and 0.09% sodium azide) may be used.

(7) Analyze cells on flow cytometer
(a) tubes 1-5 are to be used as controls to set up cytometer (i.e Compensation)

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
561527	Accutase™ Cell Detachment Solution	100 mL	(none)





***Top**: Schematic workflow of the human MSC analysis kit.*
***Bottom:** Cells grown in BD Mosaic™ hMSC SF Cell Culture Environment (Cat. No. 355700) were detached using BD™ Accutase™ Cell Detachment Solution (Cat. No 561527) and then stained with the positive and negative cocktails (solid lines) or the positive and negative isotype control cocktails (dashed lines). The plots were derived from gated events based on light scattering characteristics of the MSCs. Cells were analyzed using a BD™ LSRII flow cytometry system.*

Product Notices

1. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
2. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
3. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
4. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

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5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
6. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
7. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Sánchez L, Gutierrez-Aranda I, Ligero G, et al. Enrichment of Human ESC-Derived Multipotent Mesenchymal Stem Cells with Immunosuppressive and Anti-Inflammatory Properties Capable to Protect Against Experimental Inflammatory Bowel Disease. *Stem Cells.* 2010; 29(2):251-262. (Biology: Cell differentiation)

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