

BD Human Mesenchymal Stromal Cell Kit and Template

hMSC Phenotyping on the BD Accuri™ C6 Flow Cytometer

Features

Preconfigured kit, protocol, and software template to characterize human bone marrow-derived MSCs on the BD Accuri C6

Support studies involving hBM-MSc positive markers CD105, CD73, and CD90, and negative markers CD45, CD34, CD11b, CD19, and HLA-DR

Enable quick and easy setup and analysis using the BD Accuri C6



The BD Stemflow™ Human MSC Analysis Kit (Cat. No. 562245), protocol, and software template for the BD Accuri™ C6 flow cytometer simplify the characterization of human bone marrow-derived mesenchymal stromal cells (hBM-MSCs). The kit, based on the phenotypic human multipotent MSC signature described by the International Society for Cellular Therapy (ISCT), includes fluorescent antibodies and controls needed for acquisition and analysis. The panel's modular design supports the simultaneous analysis of additional positive or negative markers, and includes an optional drop-in positive marker (CD44). A BD Accuri™ C6 software template matched to the kit includes a predefined workspace, markers, regions, gates, and parameter names for quick and easy setup and analysis.

Figure 1 shows data collected on the BD Accuri C6 using the preconfigured kit and software template.

Researchers who culture MSCs are concerned with their purity and multipotency. The ISCT has proposed a minimal set of three standard criteria to be used as the uniform definition of hMSCs: 1) adherence to plastic, 2) specific surface antigen expression, and 3) multipotent differentiation potential. The phenotype of hMSCs is defined, at a minimum, as 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, CD79a or CD19, and HLA-DR ($\leq 2\%$ positive).¹ Multicolor flow cytometry can be used to demonstrate that individual cells co-express unique hMSC markers and lack hematopoietic antigen expression.

The BD Stemflow hMSC Analysis Kit allows researchers to obtain precise data on the percentage of cells positively expressing the defined cell surface markers of hBM-MSCs vs contaminating hematopoietic cells from samples of near-pure or even highly heterogeneous cell populations. The kit contains corresponding isotype controls to improve productivity and reduce assay-to-assay variability, as well as single-stained cellular compensation controls to standardize instrument setup procedures.

Investigators can use additional surface markers (both positive and negative) to demonstrate higher levels of purity for certain experiments. For immunophenotypic analyses beyond the minimal requirements, the kit's modular architecture enables the easy addition of supplementary monoclonal antibodies against critical cell surface markers. The positive MSC cocktail leaves the PE channel open to use with either the negative MSC PE cocktail, the drop-in CD44 PE antibody conjugate (both included in the kit), or any other PE antibody conjugate.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and a nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.

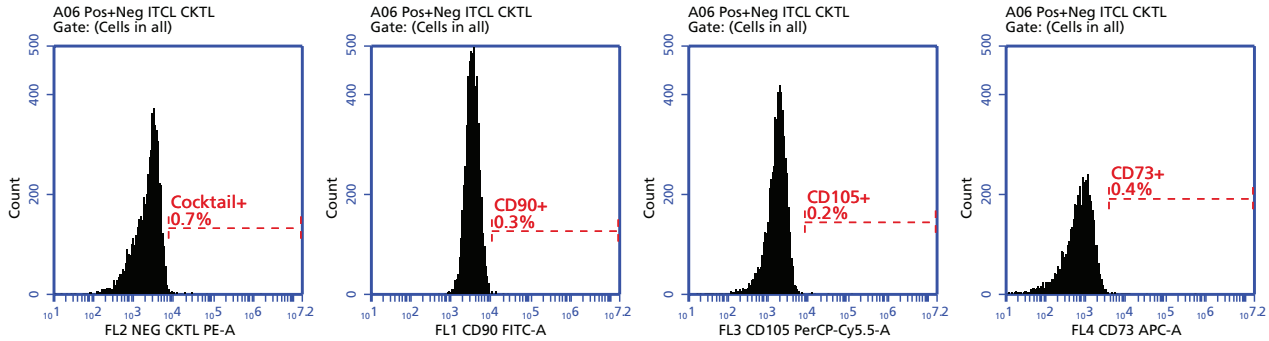
Visit bdbiosciences.com for more information.

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BD Human Mesenchymal Stromal Cell Kit and Template

A Isotype Controls



B hMSC Cocktails

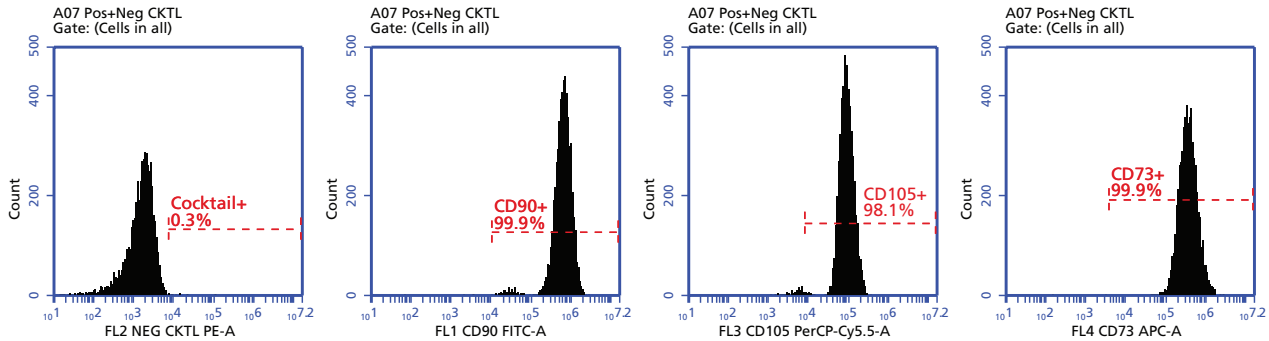


Figure 1. BD Stemflow™ Human MSC Analysis Kit (Cat. No. 562245) analysis on the BD Accuri C6.

hBM-MSCs (Lonza) were disassociated using BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527), stained according to kit instructions, and acquired on a BD Accuri C6 using the kit template. Cells were gated on light scatter properties of hBM-MSCs and analyzed for expression of key hBM-MSC surface markers using BD Accuri C6 software. **Results:** A. Gates were drawn based on matched isotype control cocktails. B. The vast majority of analyzed cells expressed the positive hBM-MSC surface markers in the MSC positive marker cocktail (CD90, CD105, and CD73), while very few expressed the negative surface markers in the MSC negative marker cocktail (CD34, CD11b, CD19, CD45, and HLA-DR).

Ordering Information

All kits and their associated software templates are available at bdbiosciences.com/go/templates.

Description	Clone	Quantity	Number of Tests	Cat. No.	
BD Stemflow™ Human MSC Analysis Kit containing:					
hMSC Positive Cocktail	CD90 FITC	5E10	20 µL	50 tests	562245
	CD105 PerCP-Cy™5.5	266			
	CD73 APC	AD2			
hMSC Negative Cocktail	CD34 PE	581	20 µL		
	CD11b PE	ICFR44			
	CD19 PE	HIB19			
	CD45 PE	HI30			
	HLA-DR PE	G46-6			
CD44 PE (optional positive drop-in, 100 tests)	G44-26	5 µL			
Isotype controls as detailed in technical data sheet					
Compensation controls as detailed in technical data sheet					

Related Kits

Description	Cat. No.
BD Stemflow™ Human Mesenchymal Stem Cell Lineage Antibody Cocktail	562530

References

- 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.

Class 1 Laser Product.

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