

BD LSRFortessa™ X-20 System

Eight-Color Research Panel Designed for the Identification of Human Mesenchymal Stromal Cell Subpopulations

In these experiments, a five-laser special order BD LSRFortessa™ X-20 system was used in combination with BD reagents to design multicolor flow cytometry panels. The BD Stemflow™ Human MSC Analysis Kit and additional antibodies conjugated with BD Horizon Brilliant™ Ultraviolet and Violet fluorochromes were used to identify mesenchymal stromal cell (MSC) subpopulations.

Analyzer Configuration

Laser	Filter	Fluorochrome	Panel	Cat. No.
Blue 488 nm	530/30	FITC	CD90	562245 (BD Stemflow Human MSC Analysis Kit)
	695/40	PerCP-Cy™5.5	CD105	
Red 640 nm	670/30	APC	CD73	
Yellow-green 561 nm	586/15	PE	Lineage cocktail	
Violet 405 nm	450/40	BV421	CD271	562562
	525/50	BV510	GD2	563440
	710/50	BV711	CD146	563186
Ultraviolet 355 nm	379/28	BUV395	SSEA-4	563817

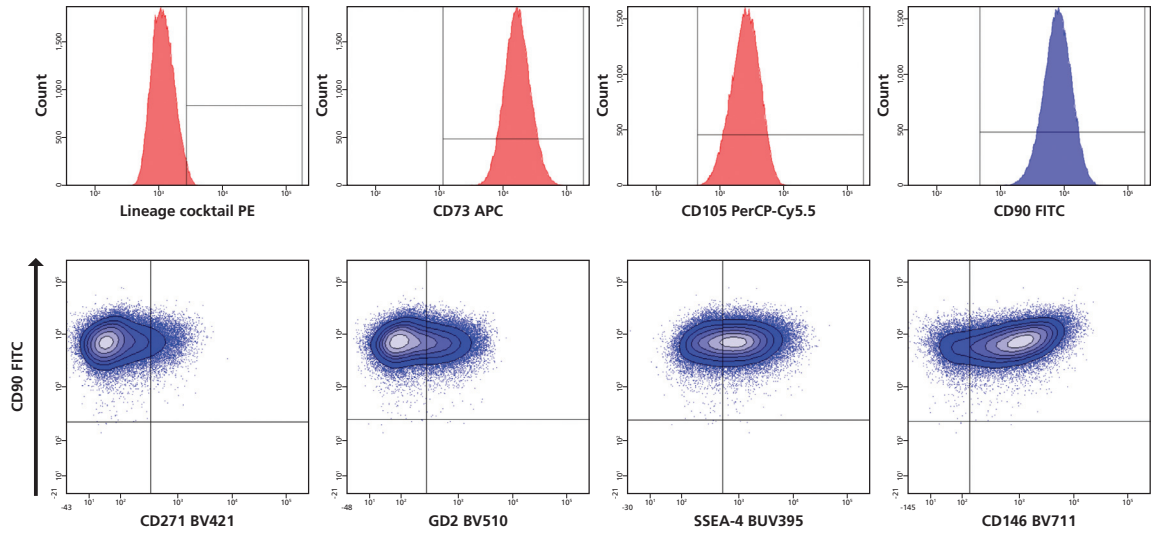


Protocol

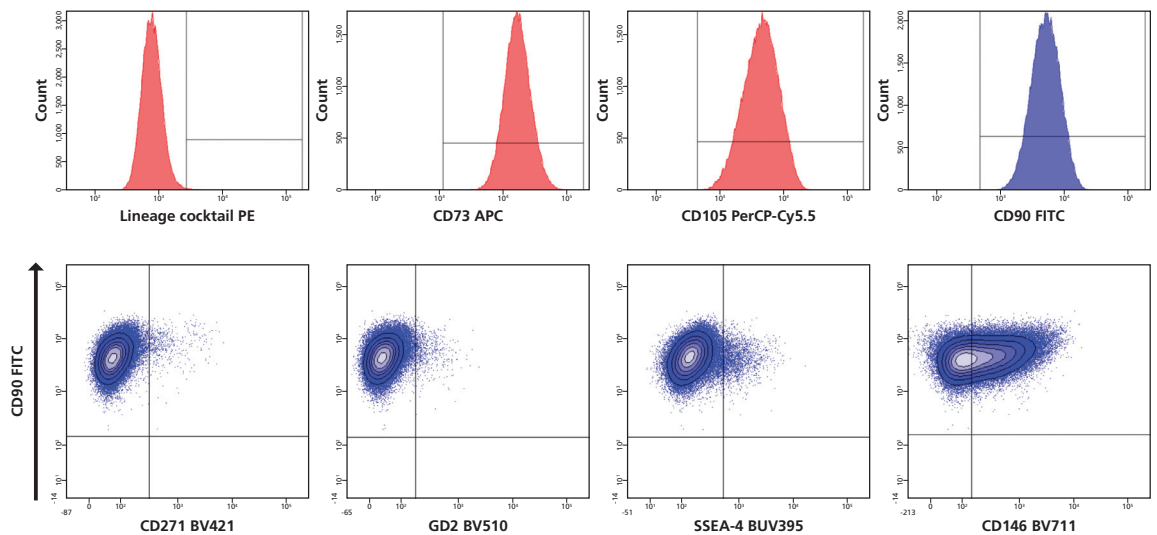
Human MSCs derived from bone marrow or adipose tissue (BM MSCs and ADSCs, Lonza) were analyzed at passage 5 of culture. Cells were detached using BD Accutase™ Cell Detachment Solution (Cat. No. 561527). Cells were then incubated with antibodies and BD Horizon Brilliant™ Stain Buffer (Cat No. 563794) at room temperature for 30 minutes, washed, and acquired on a BD LSRFortessa X-20 flow cytometer.

Data

A: BM MSCs



B: ADSCs



Discussion

Human MSCs are conventionally defined based on the homogeneous expression of CD90, CD73, CD105, and lack of hematopoietic lineage markers.¹ Recent studies have demonstrated the existence of subpopulations of human MSCs characterized by the expression of SSEA-4, CD146, GD2, or CD271.²⁻⁵ To analyze the expression of these subpopulations, BM MSCs were stained with the BD Stemflow Human MSC Analysis Kit. Additional antibodies, SSEA-4 BD Horizon Brilliant™ Ultraviolet 395 (BUV395), CD271 BD Horizon Brilliant™ Violet 421 (BV421), GD2 BD Horizon Brilliant™ Violet 510 (BV510), and CD146 BD Horizon Brilliant™ Violet 711 (BV711), were added to the kit. (A): The purity of human MSCs was determined by the expression of CD90, CD73, CD105, and lack of expression of hematopoietic lineage markers. After gating on CD90⁺ cells, subpopulations of BM MSCs could be simultaneously identified based on the expression of CD271, GD2, SSEA-4, and CD146. (B): Similar to BM MSCs, ADSCs homogeneously expressed CD73, CD105, and CD90, and lacked the expression of hematopoietic lineage markers. However, further analysis of CD271, GD2, SSEA-4, and CD146 revealed a different profile of expression of the antigens compared to BM MSCs.

Conclusion

The BD Stemflow Human MSC Analysis Kit can be used to identify MSCs based on the minimal criteria as previously reported.¹ For a more extensive characterization, BD Horizon Brilliant Ultraviolet and Violet reagents can be multiplexed with the BD Stemflow Human MSC Analysis Kit to identify MSC subpopulations.

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.
2. Rosu-Myles M, McCully J, Fair J, et al. The globoseries glycosphingolipid SSEA-4 is a marker of bone marrow-derived clonal multipotent stromal cells in vitro and in vivo. *Stem Cells Dev*. 2013;22:1387-1397.
3. Espagnolle N, Guilloton F, Deschaseaux F, Gadelorge M, Sensébé L, Bourin P. CD146 expression on mesenchymal stem cells is associated with their vascular smooth muscle commitment. *J Cell Mol Med*. 2014;18:104-114.
4. Xu J, Liao W, Gu D, et al. Neural ganglioside GD2 identifies a subpopulation of mesenchymal stem cells in umbilical cord. *Cell Physiol Biochem*. 2009;23:415-424.
5. Kuçi S, Kuçi Z, Kreyenberg H, et al. CD271 antigen defines a subset of multipotent stromal cells with immunosuppressive and lymphohematopoietic engraftment-promoting properties. *Haematologica*. 2010;95:651-659.

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Cy™ is a trademark of GE Healthcare. Cy™ dyes are subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and are made and sold under license from GE Healthcare only for research and in vitro diagnostic use. Any other use requires a commercial sublicense from GE Healthcare, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA.

BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2014 BD
23-16187-00

