

# 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

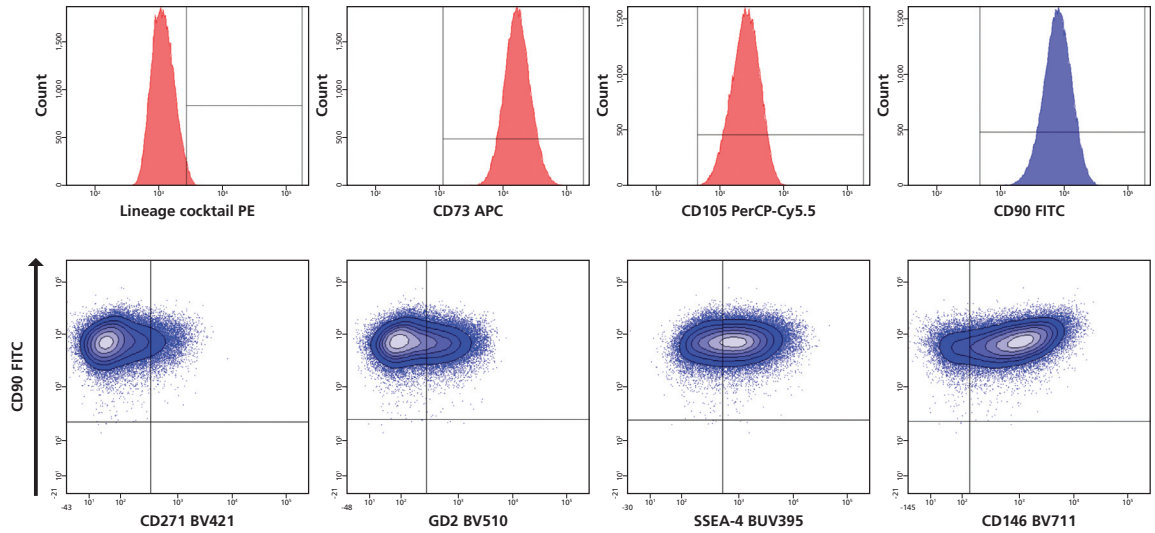


**Protocol**

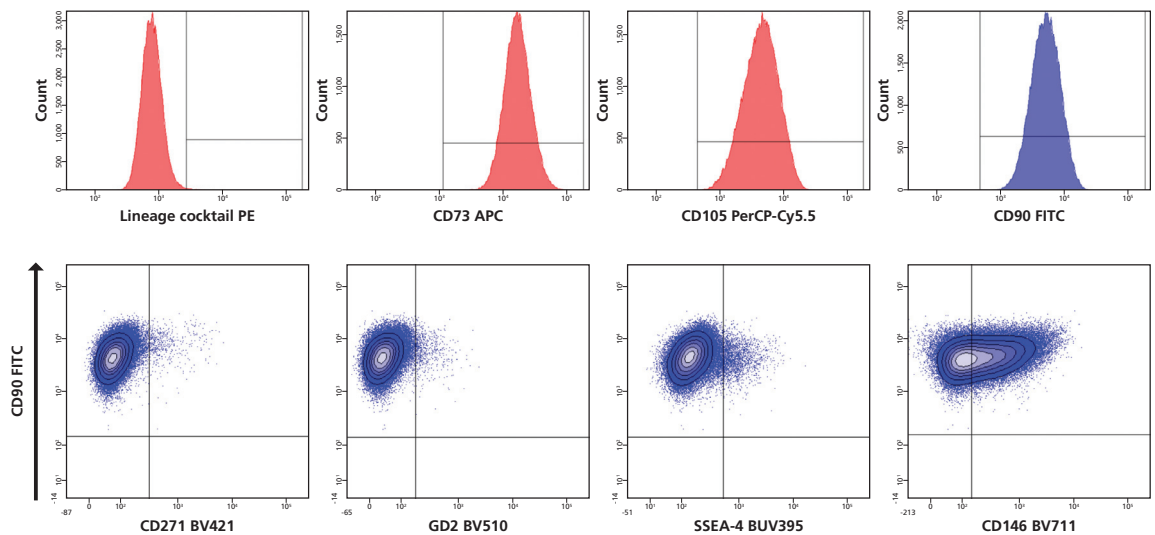
Human MSCs derived from bone marrow or adipose tissue (BM MSCs and ADSCs, Lonza) were analyzed at passage 5 of culture. Cells were detached using BD Accutase™ Cell Detachment Solution (Cat. No. 561527). Cells were then incubated with antibodies and BD Horizon Brilliant™ Stain Buffer (Cat No. 563794)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

**Data**

**A: BM MSCs**



**B: ADSCs**



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## Discussion

Human MSCs are conventionally defined based on the homogeneous expression of CD90, CD73, CD105, and lack of hematopoietic lineage markers.<sup>1</sup> Recent studies have demonstrated the existence of subpopulations of human MSCs characterized by the expression of SSEA-4, CD146, GD2, or CD271.<sup>2-5</sup> 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<sup>+</sup> 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.

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## Conclusion

The BD Stemflow Human MSC Analysis Kit can be used to identify MSCs based on the minimal criteria as previously reported.<sup>1</sup> 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.

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## 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.

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