BD LSRFortessa™ X-20 System

Four-Color Research Panels Designed for the Characterization of Stem Cells with Minimal Spectral Overlap

In these experiments, a BD LSRFortessa™ X-20 system was used in combination with BD reagents to design panels that are optimized for minimal spectral overlap by selecting one fluorochrome per laser. Two different panels are shown: human mesenchymal stromal cells (hMSCs) and human embryonic stem cells (hESCs).

Analyzer Configuration

Laser	Filter	Fluorochrome	hMSC Panel	Cat. No.
Blue 488 nm	530/30	FITC	CD90	555595
Red 640 nm	670/30	Alexa Fluor® 647	CD146	563619
Violet 405 nm	450/40	BV421	CD271	562562
Ultraviolet 355 nm	379/34	BUV395	SSEA-4	563817

Laser	Filter	Fluorochrome	hESC Panel	Cat. No.
Blue 488 nm	530/30	FITC	TRA-1-60	560380
Red 640 nm	670/30	Alexa Fluor® 647	SSEA-1	560120
Violet 405 nm	450/40	BV421	SSEA-3	562706
Ultraviolet 355 nm	379/34	BUV395	SSEA-4	563817

Compensation*

Fluorochrome	(-)% Fluorochrome	% Compensation
FITC	BV421	0.01%
Alexa Fluor® 647		0.00%
BUV395		0.00%
BV421	FITC	0.02%
Alexa Fluor® 647		0.00%
BUV395		0.00%
BV421	Alexa Fluor® 647	0.00%
FITC		0.00%
BUV395		0.00%
BV421	BUV395	0.12%
FITC		0.00%
Alexa Fluor® 647		0.00%

*Representative compensation values. Compensation varies as a function of photomultiplier tube voltage.

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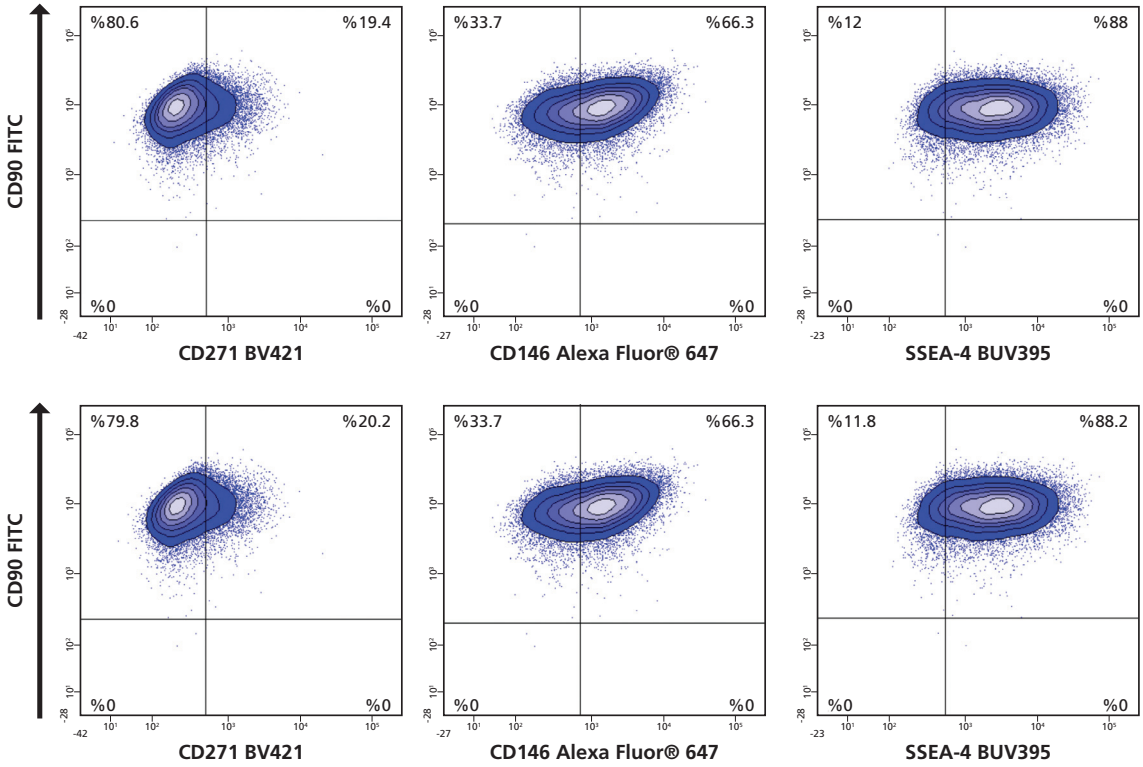
Protocol

Human bone marrow-derived MSCs (Lonza) were analyzed at passage 5 of culture. Cells were harvested 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. The purity of the hMSCs was previously determined using the BD Stemflow™ Human MSC Analysis Kit (Cat. No. 562245).

H9 hESCs (WiCell, Madison, WI) grown in mTeSR™1 medium (StemCell Technologies) were harvested with BD Accutase Cell Detachment Solution. Cells were then incubated with antibodies and BD Horizon Brilliant Stain Buffer at room temperature for 30 minutes, washed, and acquired on a BD LSRFortessa X-20 flow cytometer.

Data

Analysis of hMSCs



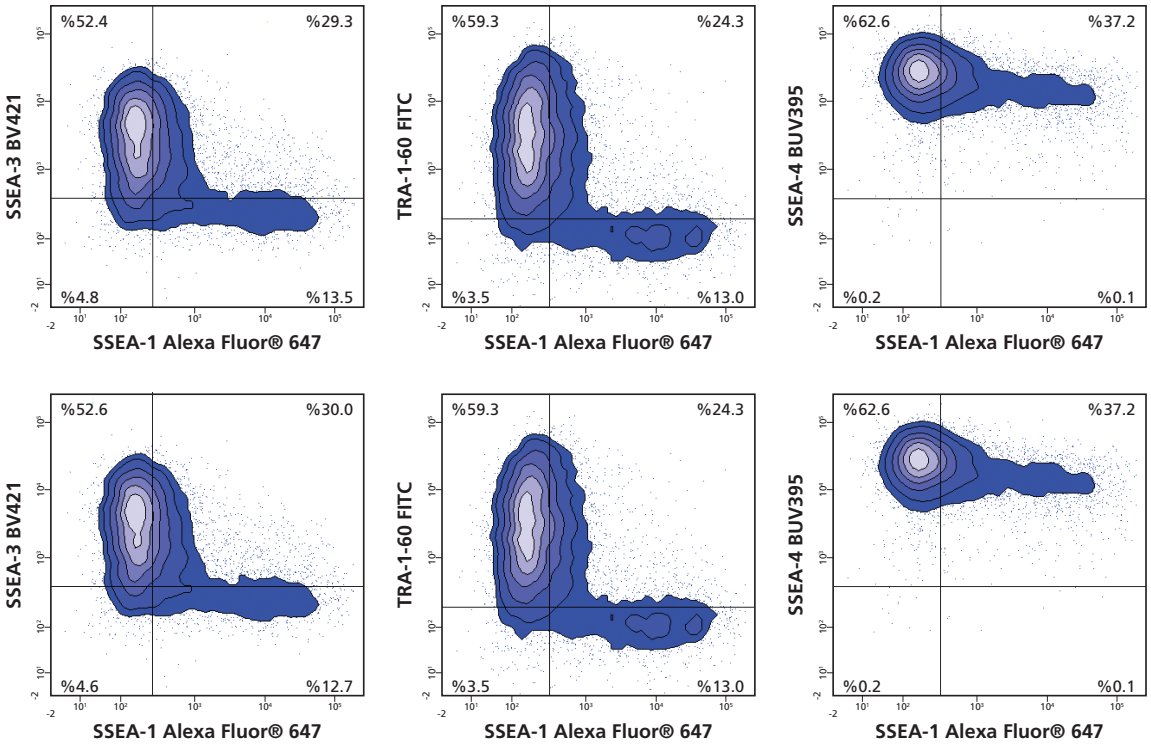
Discussion

Recent studies have demonstrated the existence of subpopulations of hMSCs characterized by the expression of SSEA-4, CD146, or CD271.[1-3] To analyze the expression of these subpopulations, cells were stained with CD90 FITC, SSEA-4 BD Horizon Brilliant™ Ultraviolet 395 (BUV395), CD146 Alexa Fluor® 647, and CD271 BD Horizon Brilliant™ Violet 421 (BV421). Singlet cells were discriminated based on light scatter properties, and hMSCs were identified based on the expression of CD90, one of the hallmark markers homogeneously expressed by MSCs.[4] Within the $CD90^+$ population it is possible to simultaneously identify multiple subpopulations of hMSCs expressing SSEA-4, CD146, and CD271.

Compensated data (upper panel) and uncompensated data (lower panel) show similar staining profiles.

Data

Analysis of hESCs



Discussion

H9 hESCs (WiCell, Madison, WI) were stained with SSEA-4 BUV395, SSEA-3 BV421, TRA-1-60 FITC, and SSEA-1 Alexa Fluor® 647. Singlet hESCs were discriminated by light scatter. The expression of pluripotency markers SSEA-4, SSEA-3, and TRA-1-60, and also the differentiation marker SSEA-1, was analyzed.[5,6]

Compensated data (upper panel) and uncompensated data (lower panel) show similar staining profiles.

Conclusion

The laser configuration of the BD LSRFortessa X-20 system, combined with novel BD Horizon Brilliant Violet and BD Horizon Brilliant Ultraviolet reagents, enables new optimal panel design. The compensation on a data set can be minimized by the use of dyes with minimal spectral overlap as well as an optimized cytometer configuration.

References

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3. 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.
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5. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002;200:249-258.
6. Adewumi O, Aflatoonian B, Ahrlund-Richter L, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol*. 2007;25:803-816.