

# Detection of human mesenchymal stem cells

## Using the Attune® Acoustic Focusing Cytometer

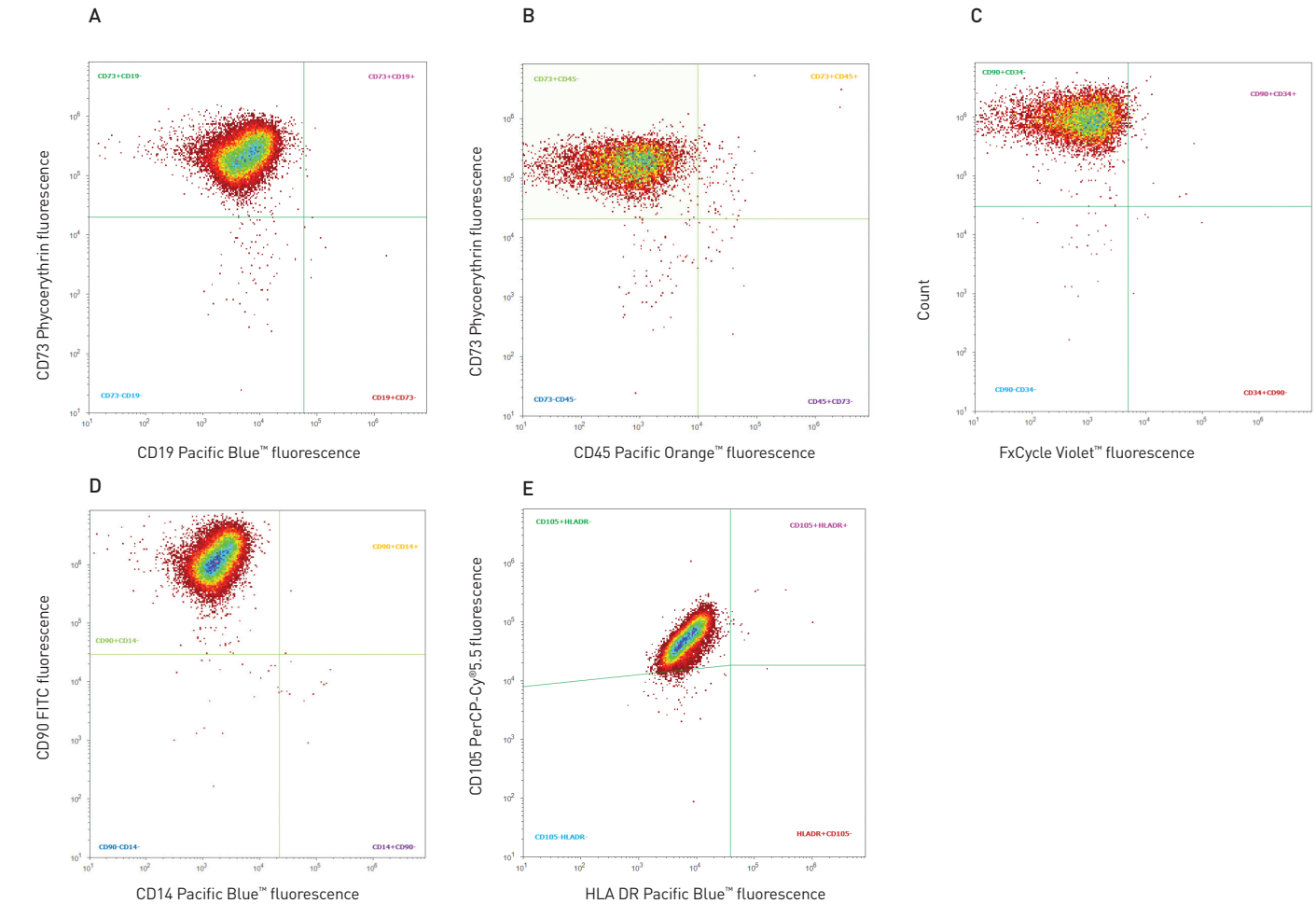
Adult human mesenchymal stem cells (hMSCs) are rare fibroblast-like cells capable of differentiating into a variety of cell tissues, including bone, cartilage, muscle, ligament, tendon, and adipose. The International Society for Cellular Therapy (ISCT) has proposed a set of standards to define hMSCs for laboratory investigations and preclinical studies: adherence to plastic in standard culture conditions; *in vitro* differentiation into osteoblasts, adipocytes, and chondroblasts; and specific surface antigen expression in which  $\geq 95\%$  of the cells express the antigens recognized by CD105, CD73, and CD90, with the same cells lacking ( $\leq 2\%$  positive) the antigens recognized by CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR [1]. Recent studies have shown that CD34 antigen may be present, but its expression is transient and present only in early passages of cells derived from some isolates. Direct measurement of proliferation combined with simultaneous detection of the ISCT-consensus immunophenotypic profile provides data that are used to determine the differentiation status and health of the cells [2]. Here we show two examples—immunophenotyping and cell cycle analysis.

### Materials and methods

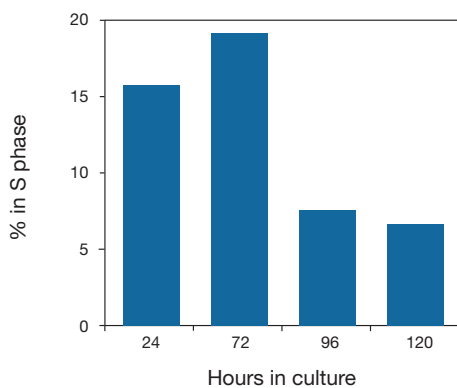
hMSCs (derived from normal human bone marrow) at passage 7 were fixed with 2% formaldehyde, blocked using normal mouse serum, stained in three tubes for 30 minutes at room temperature in the dark, and washed and suspended in PBS. Compensation was set using the AbC™ Anti-Mouse Bead Kit (Invitrogen Cat. No. A10344). Fluorescence-minus-one (FMO) controls were used for gate placement. Samples were then acquired on the Attune® cytometer using a 405 nm laser with a 450/40 bandpass and 603/48 bandpass, and a 488 nm laser with a 530/30 bandpass, 575/24 bandpass, and 640 longpass.

The panel configurations are as follows:

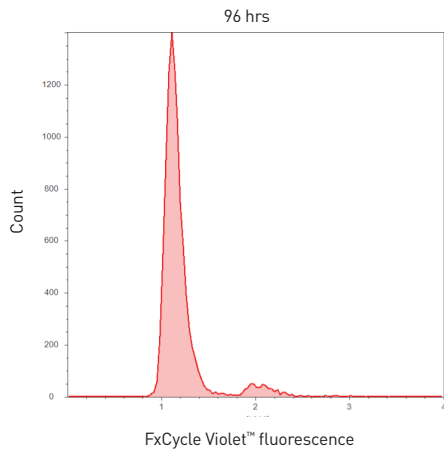
- CD73 PE, CD19 Pacific Blue™, CD45 Pacific Orange™ direct conjugates
- CD90 FITC, CD34 PE, CD14 Pacific Blue™ direct conjugates
- CD105 PerCP-Cy®5.5, HLA-DR Pacific Blue™ direct conjugates



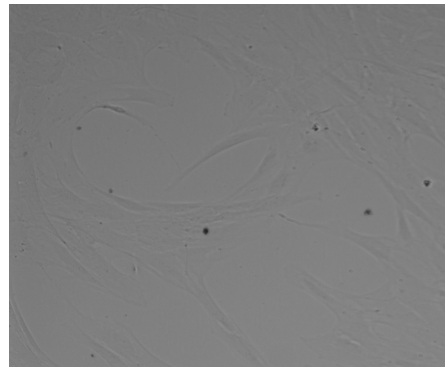
**Figure 1. Immunophenotype data.** Human mesenchymal stem cells collected at passage 7 were identified as: **(A)** 99.7% CD73<sup>+</sup>/0.29% CD19<sup>-</sup>; **(B)** 99.4% CD73<sup>+</sup>/0.22% CD45<sup>-</sup>; **(C)** 99.7% CD90<sup>+</sup>/0.09% CD34<sup>-</sup>; **(D)** 99.9% CD90<sup>+</sup>/0.07% CD14<sup>-</sup>; **(E)** 99.2% CD105<sup>+</sup>/0.76% HLA-DR<sup>-</sup>. The data demonstrate that cell expression remains as expected after 7 passages.



**Figure 2. Undifferentiated hMSCs, passage 7 (hMSC P7), demonstrate a decrease in percentage in S phase as the cells remain in culture and become more confluent over time.** Subculturing prior to 80% confluency or 3–5 days is suggested for an optimized growth rate without differentiation. After 72 hr, the percentage in S phase is reduced significantly, revealing a decrease in DNA replication and indicating cells should be subcultured according to recommended criteria. At 0 hr, hMSC P7 were plated into separate tissue culture dishes with DMEM, 10% hMSC FBS, and 2 mM L-glutamine. Cells were harvested using TrypLE™ Express at 24, 72, 96, and 120 hr, fixed in 70% EtOH, and stored at -20°C. For analysis, cells were washed and resuspended in DPBS with 0.1% Triton® X-100. Cells were adjusted to a concentration of 10<sup>5</sup>/mL using the Countess® Automated Cell Counter and labeled with 500 nM FxCycle™ Violet stain. Samples were analyzed on the Attune® Acoustic Focusing Cytometer, and ModFit LT™ (Verity Software House) curve-fitting software was used to extract the cell cycle phase distributions.



**Figure 3. All phases of the cell cycle detected in hMSCs using FxCycle™ Violet stain and the Attune® Acoustic Focusing Cytometer.** Cells were analyzed at 96 hr without subculturing. A significant decrease in percentage of cells in S phase as culture time is extended indicates a reduction in growth rate and emphasizes the need for earlier subculturing to optimize growth rate and nondifferentiation.



**Figure 4. 10x bright-field image of hMSCs at 96 hr prior to harvesting, using an EVOS® microscope.** Cells are near 100% confluency. Further analysis of cell cycle distribution shows a reduced percentage of cells in S phase, compared to prior harvest times.

## References

1. Dominici M, Le Blanc K, Mueller I et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315–317.
2. Bradford JA, Clarke ST (2011) Panel Development for Flow Cytometry Testing of Proliferation and Immunophenotype in hMSCs. In Vemuri MC, Chase LG, Rap MS, *Mesenchymal Stem Cell Assays and Applications* (2011, pp. 367–385). New York: Springer.

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