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tion because it limits multicolor analysis: FL1 emission is used for nucleic acid staining, FL2 (FL4 for dual-laser analysis), for CD34 staining, and FL3, for CD45 staining, leaving only one fluorescence parameter available for further characterization of CD34+ cells. Other limitations are related to the staining procedure. A decreased mean fluorescence intensity for APC-CD34, when compared with PE-CD34 staining, can complicate APC-CD34+ cell gating in relation to the very close non-CD34-expressing cells. Inappropriate compensation for the fluorescence wavelength overlap of PC5 and APC can result in decreased CD34+ counts. Counting total nucleated cells using SYTO-13 could make CD45 staining unnecessary and leave FL3 available for better characterization of CD34+ cells. The development of more-specific DNA dyes for vital cell staining could contribute to efficient resolution of the region of nucleated cells, and consequently improve immunophenotyping of whole blood with single-laser instruments. More-sensitive measurements of CD34+ events have increasing clinical relevance: They could have an effect on research in a broad range of scientific and clinical disciplines, and could also clarify the number of CD34 cells needed for engraftment.

Table 1 Limits of agreement for method comparisons

	ProCOUNT vs. SYTO	SYTO-L vs. SYTO	ProCOUNT vs. SYTO -L
BM (n = 20)	–59.41 ± 345.05 (–220.90 – 102.08)	64.65 ± 379.62 (–113.02 – 242.32)	–124.06 ± 477.11 (–347.36 – 99.23)
PB (n = 20)	12.13 ± 25.82 (0.04 – 24.21)	14.13 ± 22.03 (3.82 – 24.44)	–2.00 ± 12.33 (–7.77 – 3.77)
Aph (n = 20)	385.68 ± 410.47 (193.57 – 577.79)	337.79 ± 388.07 47.89 ± 215.65	(156.17 – 519.42) (–55.04 – 148.82)
CB (n = 20)	12.38 ± 17.47 (4.21 – 20.56)	6.17 ± 14.36 (0.55 – 12.90)	6.21 ± 15.11 (–0.86 – 13.28)

Absolute CD34+ counts in bone marrow (BM), peripheral blood (PB), apheresis (Aph) and cord blood (CB). Results are expressed as the mean difference and the variability (average ± standard deviation of the difference). In parentheses, lowest and highest values for the 95% confidence interval.

Acknowledgments

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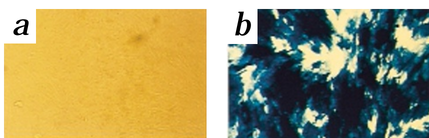
ON THE MARKET

ADENOVIRAL SYSTEM



Adeno-X efficiently expresses β-galactosidase in human primary fibroblasts (BJ cells) (***a***), noninfected BJ cells (***b***). BJ cells were infected for 30 min with unpurified recombinant adenovirus (104 pfu/ml) containing pAdeno-lacZ. Cells were cultured under normal conditions for 48 h, then fixed and stained for β-galactosidase expression.

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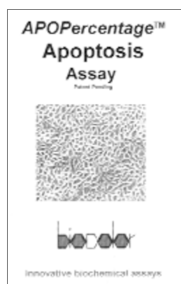

Getting kitted out with the APOPercentage apoptosis assay.

APOPercentage is Accurate Chemical & Scientific's dye-specific **detection and quantification system to monitor apoptosis in mammalian cells** during *in vitro* culture. A specific purple-red dye is used to label cells that are undergoing apoptosis. The company says that an individual apoptotic cell, or even an intact isolated 'bleb', can be observed within the cellular monolayer containing up to 50,000 cells, as present in a single well. The assay does not require fluorescent-labeled antibodies or enzymes.

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The polyMICA detection kit from The Binding Site is intended for the **immunohistochemical detection of antigens in human tissue sections** that have first been labeled with primary antibodies raised in sheep or mouse. An acronym for polyclonal mirror image complimentary antibodies, the principle of the polyMICA system is the sequential binding of two mutually attractive antibodies. This mutual attraction increases the valency of the antibodies when compared to traditional multi-step labeling methods, thereby increasing the sensitivity, says the company. This is said to enable the detection of many low-level target antigens, as well as offering the possibility of diluting primary antibodies 10–20 times further, or greater. The polyMICA system is biotin-free and, as such, avoids the non-specific staining of endogenous biotin.

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M-MLV reverse transcriptase, RNase H Minus from Promega is an **RNA-dependent DNA polymerase enzyme** that can be used in cDNA synthesis with long messenger RNA templates (>5 kilobases). Other applications include RT-PCR. The lack of RNase H activity is germane to the applications in that this activity can start to degrade templates and first-strand products when incubation times are increased for larger cDNA products. The enzyme is available in catalog package sizes, as well as in bulk quantities.

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