BD Accuri™ C6 and BD Pharmingen™ Apoptosis, DNA Damage and Cell Proliferation Kit:
Monitoring cellular processes in stem cell preparations

This experimental data demonstrates the characterization of three different types of stem cell preparations for their apoptotic, proliferative, and DNA damage status.

Experiment 1

H9 hESCs

H9 human embryonic stem cells (hESCs) were dissociated and analyzed for expression of a key pluripotency marker (Nanog), in combination with markers to detect proliferating cells (BrdU), apoptotic cells (cleaved PARP), and cells with DNA damage (phosphorylated H2AX). Data was collected and analyzed on a BD Accuri™ C6 personal flow cytometer. All data was gated based on the light scatter properties of H9 hESC.

Material

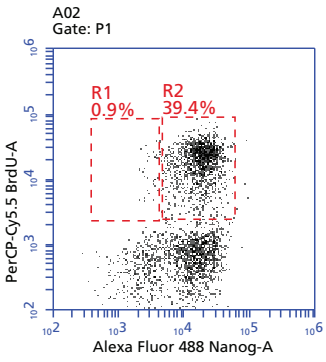
Reagents

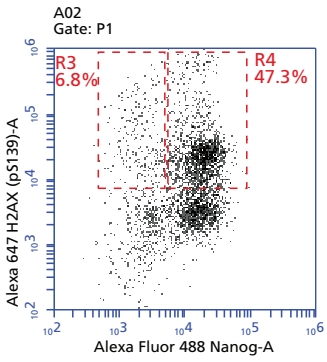
BD Pharmingen™ Alexa Fluor® 488 anti-Nanog antibody (Cat. No. 560791)
BD Pharmingen Apoptosis, DNA Damage and Cell Proliferation Kit (Cat. No. 562253), including BrdU, anti-BrdU, anti cleaved PARP, and anti phosphorylated H2AX (pS139) antibodies

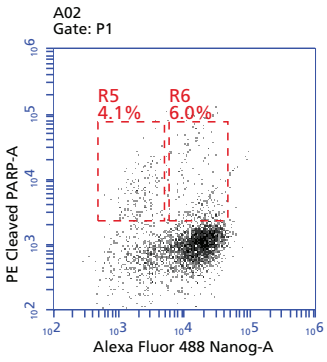
Additional Material

BD Matrigel™ hESC-qualified matrix (Cat. No. 354277)
BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527)

Data







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Experiment 2

Neural ectoderm cultures

H9 hESC derived neural ectoderm cultures[1,2] were analyzed for expression of the neural stem cell (NSC) marker Sox2, in combination with markers to detect proliferating cells (BrdU), apoptotic cells (cleaved PARP), and cells with DNA damage (phosphorylated H2AX). Data was collected and analyzed on a BD Accuri™ C6 personal flow cytometer. All data was gated based on the light scatter properties of H9 hESC derivatives.

Material

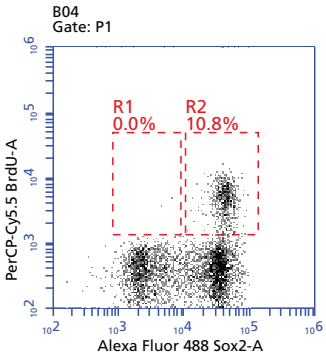
Reagents

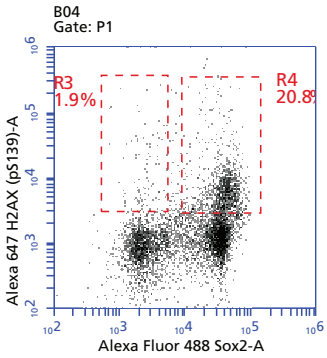
BD Pharmingen™ Alexa Fluor® 488 anti-Sox2 antibody (Cat. No. 561593)
BD Pharmingen Apoptosis, DNA Damage and Cell Proliferation Kit (Cat. No. 562253), including BrdU, anti-BrdU, anti cleaved PARP, and anti phosphorylated H2AX (pS139) antibodies

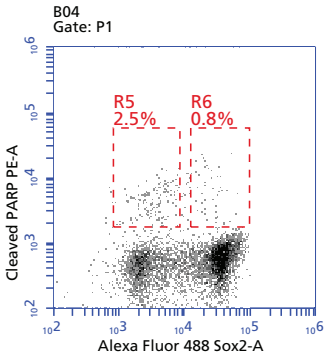
Solution

BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527)

Data







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Experiment 3

Definitive endoderm cultures

H9 hESC definitive endoderm cultures[3] were analyzed for expression of the definitive endoderm differentiation marker Sox17, in combination with markers to detect proliferating cells (BrdU), apoptotic cells (cleaved PARP), and cells with DNA damage (phosphorylated H2AX). Data was collected and analyzed on a BD Accuri C6 personal flow cytometer. All data was gated based on the light scatter properties of H9 hESC derivatives.

Material

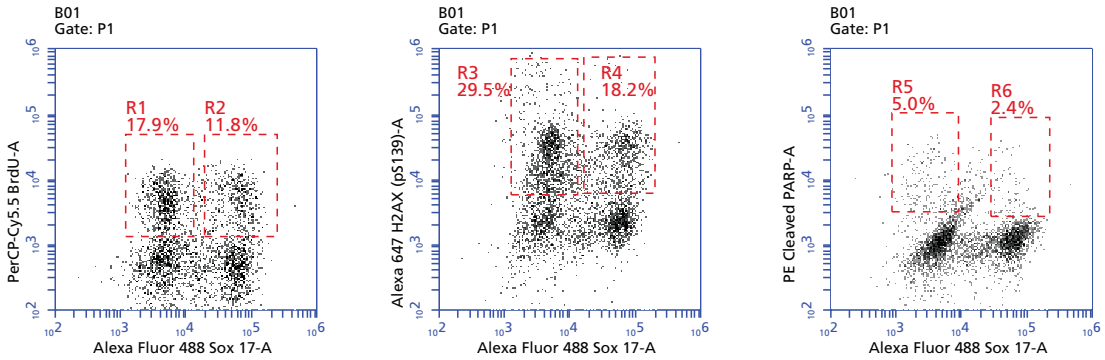
Reagents

BD Pharmingen™ Alexa Fluor® 488 anti-Sox17 antibody (Cat. No. 562205)
BD Pharmingen Apoptosis, DNA Damage and Cell Proliferation Kit (Cat. No. 562253), including BrdU, anti-BrdU, anti cleaved PARP, and anti phosphorylated H2AX (pS139) antibodies

Solution

BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527)

Data



Discussion

The data demonstrates that rapid and multiparametric flow cytometry using the BD Accuri C6 personal flow cytometer, and BD Pharmingen reagents and kits is effective for monitoring proliferation, DNA damage, and apoptosis in specific subpopulations of heterogeneous stem cell preparations.

References

1. Yuan SH, Martin J, Elia J, et al. Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells. *PloS One*. 2011;6:e17540.

2. Zhou J, Su P, Li D, Tsang S, Duan E, Wang F. High-efficiency induction of neural conversion in hESCs and hiPSCs with a single chemical inhibitor of transforming growth factor beta superfamily receptors. *Stem Cells*. 2010;28:1741-1750.

3. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol*. 2005;23:1534-1541.

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