BD Accuri™ C6 and BD Pharmingen™ Stem Cell Marker Antibodies:

Monitoring human embryonic stem cell differentiation to neural ectoderm

This experimental data demonstrates the characterization of cells from a neural induction culture using key neural differentiation markers and established assays for apoptosis, proliferation, and DNA damage.

Experiment 1

H9 hESCs were differentiated to neural ectoderm for 6 weeks as described in previous methods.[1,2] Cells were dissociated and analyzed for expression of key neural differentiation markers: Sox1, Sox2, and Pax-6. Data was collected and analyzed on a BD Accuri™ C6 personal flow cytometer. All data shown was gated based on the light scatter properties of H9 hESC derivatives.

Material

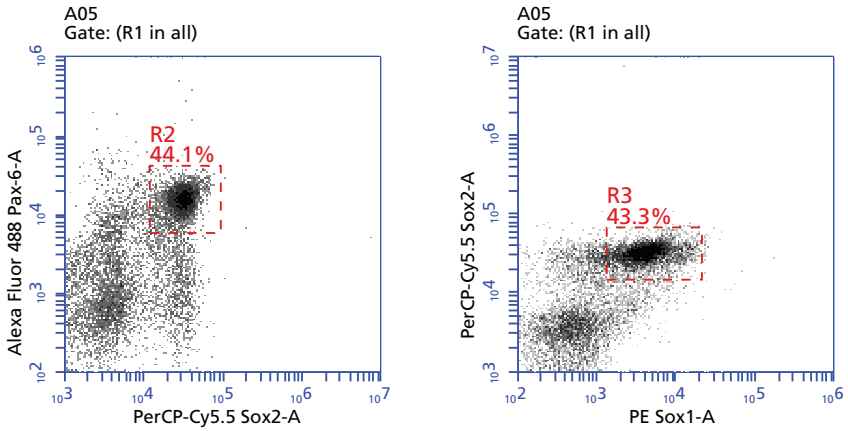
Reagents

BD Pharmingen™ Alexa Fluor® 488 anti-Pax6 antibody (Cat. No. 561664)
BD Pharmingen PE anti-Sox1 antibody (Cat. No. 561592)
BD Pharmingen PerCP-Cy™5.5 anti-Sox2 antibody (Cat. No. 561506)

Buffers and Solutions

BD Cytofix™ fixation buffer (Cat. No. 554655)
BD Phosflow™ perm buffer III (Cat. No. 558050)
BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527)

Data



Experiment 2

Cells from a neural induction culture as described in Experiment 1 were dissociated and analyzed for expression of the hNSC 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 shown was gated based on the light scatter properties of H9 hESC derivatives.

Material

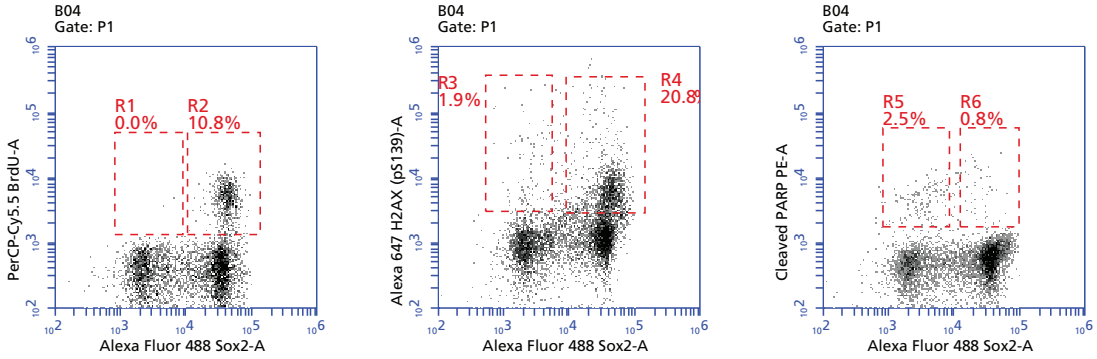
Reagents

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



Discussion

The data demonstrates that rapid and multiparametric flow cytometry using the BD Accuri C6 personal flow cytometer and BD Pharmingen antibodies and analysis kits is effective for characterization of the heterogeneous populations of cells in a neural induction culture.

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.