BD Stemflow™ Human iPSC Sorting and Analysis Kit

Features

Enables sorting and analysis of induced pluripotent stem cells

Delivers a streamlined solution for consistent experimental results

Facilitates instrument setup with BD™ CompBead Plus compensation particles and gating with isotype controls

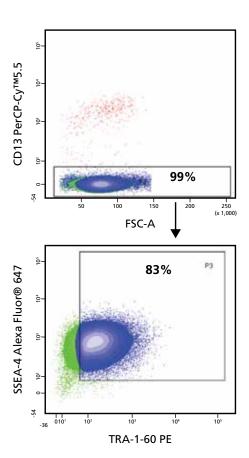


Figure 1. Established human iPSCs were stained with CD13 PerCP-Cy5.5, SSEA-4 Alexa Fluor® 647, and TRA-1-60 PE.

Data courtesy of David J. Kahler, New York Stem Cell Foundation

The BD StemflowTM human iPSC sorting and analysis kit provides a comprehensive solution for sorting and analysis of induced pluripotent stem cells (iPSCs). The kit includes pre-titrated antibodies for the identification of iPSCs, as well as instrument setup reagents, isotype controls, and a robust protocol for consistent results.

Induced Pluripotent Stem Cells

The discovery that adult fibroblast cells can be reprogrammed to an induced pluripotent state has transformed the fields of developmental biology and regenerative medicine. Like embryonic stem cells, iPSCs can self-renew and are able to form all three germ layers. The isolation of donor-specific iPSCs has provided new mechanisms to model and understand human disease. The 2012 Nobel Prize in medicine was awarded to Drs. John Gurdon and Shinya Yamanaka for their seminal work in this field, further underscoring the importance of iPSCs.

Analysis and Sorting by Flow Cytometry

Flow cytometry enables rapid analysis of heterogeneous cell populations at the single-cell level. After induction using established protocols, human iPSCs can be harvested using the provided protocol and analyzed using antibodies against surface markers TRA-1-60, SSEA-4, and CD13.¹ The TRA-1-60 and SSEA-4 antibodies mark the reprogrammed cells, while the CD13 antibody labels cells that still express markers of the starting fibroblast cell population—the cells that were not successfully reprogrammed.

Specificity	Format	Cell Population Identified
TRA-1-60	PE	Pluripotent stem cells
SSEA-4	Alexa Fluor® 647	Pluripotent stem cells
CD13	PerCP-Cy™5.5	Fibroblasts

For applications where a pure population of pluripotent cells is required, these markers can also be used to establish criteria for fluorescence activated cell sorting (FACS).

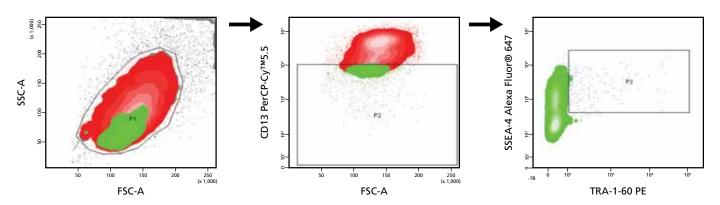
Instrument Setup and Control Reagents

BD CompBead Plus particles simplify experimental setup by facilitating compensation setup for multicolor analysis. Using beads for this purpose significantly reduces the number of cells required for each experiment while ensuring proper experimental setup. Matched isotype controls provide a means of determining background staining and establishing gates.



BD Stemflow™ Human iPSC Sorting and Analysis Kit

Cells at Day 21 Post-Transduction



Cells at Day 35 Post-Transduction

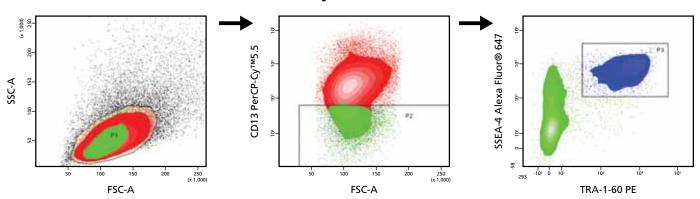


Figure 2. Human foreskin fibroblasts (Fate Therapeutics, San Diego, CA) were reprogrammed in feeder-free SMC4 small molecule conditions² and analyzed by flow cytometry using the BD Stemflow human iPSC sorting and analysis kit. Reprogrammed cells were analyzed at day 21 post-transduction (upper panels). CD13-SSEA-4+TRA-1-60+ cells were bulk sorted, further expanded, and re-analyzed at day 35 post-transduction (lower panels). Flow cytometry was performed on a BD FACSAria™ III cell sorter. Since cell types, reprogramming methods, and time courses can differ, optimal sorting timelines will need to be determined by the user.

Ordering Information

Description	Cat.No.
BD Stemflow Human iPSC Sorting and Analysis Kit (50 Tests)	562626

References

- 1. Kahler DJ, Ahmad FS, Ritz A, et al. Improved methods for reprogramming human dermal fibroblasts using fluorescence activated cell sorting. PLoS One. 2013;8:e59867.
- 2. Valamehr B, Abujarour R, Robinson M, et al. A novel platform to enable the high-throughput derivation and characterization of feeder-free human iPSCs. Sci Rep. 2012;2:213.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Alexa Fluor® is a registered trademark of Life Technologies Corporation.

Cy™ dyes are subject to proprietary rights of Amersham Biosciences Corp and Carnegie Mellon University and are made and sold under license from Amersham Biosciences Corp only for research and in vitro diagnostic use. Any other use requires a commercial sublicense from Amersham Biosciences Corp, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA.

