# BD Induced Pluripotent Stem Cell Kit and Template

## Analysis of Human iPSC Cultures on the BD Accuri™ C6 Flow Cytometer

### Features

- Preconfigured kit, protocol, and software template to analyze human induced pluripotent stem cell (hiPSC) cultures on the BD Accuri C6
- Support studies involving pluripotency surface markers SSEA-4 and TRA-1-60 and fibroblast surface marker CD13
- Enable quick and easy setup and analysis using the BD Accuri C6


The **BD Stemflow™ Human iPSC Sorting and Analysis Kit** (Cat. No. 562626), protocol, and software template for the BD Accuri™ C6 flow cytometer simplifies the analysis of human induced pluripotent stem cell (hiPSC) cultures reprogrammed from fibroblasts. The kit, which supports studies involving SSEA-4, TRA-1-60, and CD13, includes fluorescent antibodies and controls needed for acquisition and analysis. The panel is compatible with other markers to provide a base for studies of hiPSC pluripotency, reprogramming, and differentiation. A BD Accuri™ C6 software template matched to the kit includes a predefined workspace, markers, regions, gates, and parameter names for quick and easy setup and analysis.

Figure 1 shows data on the BD Accuri C6 using the preconfigured kit and software template.

The discovery that adult fibroblast cells can be reprogrammed to an induced pluripotent state has transformed the fields of developmental biology and regenerative medicine. Similarly to 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.

Flow cytometry offers an easy, rapid, and quantitative analysis of heterogeneous cell populations at the single-cell level. After culture and/or reprogramming using established protocols, human iPSCs can be analyzed using antibodies against the surface markers TRA-1-60, SSEA-4, and CD13.[1,2] The TRA-1-60 and SSEA-4 antibodies label the reprogrammed cells, while the CD13 antibody identifies cells that still express markers of the starting fibroblast cell population—cells that were not successfully reprogrammed.

Since cell types, reprogramming methods, and time courses can differ, researchers should determine optimal analysis timelines for their own experiments.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and a nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.

Visit **bdbiosciences.com** for more information.



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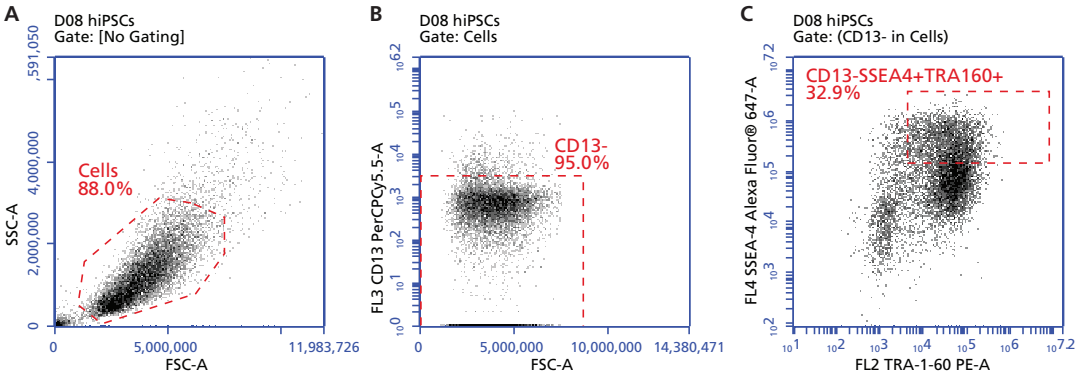


**Figure 1.** BD Stemflow Human iPSC Analysis and Sorting Kit (Cat. No. 562626) analysis on the BD Accuri C6.

Human foreskin fibroblasts (Fate Therapeutics, San Diego, CA) were reprogrammed in feeder-free SMC4 small molecule conditions.[1] Specifically, $CD13^-SSEA\text{-}4^+TRA\text{-}1\text{-}60^+$ reprogrammed cells were bulk sorted at Day 21 post-transduction, further expanded, and analyzed at Day 35 post-transduction. For the analysis, cells were disassociated using BD™ Accutase™ cell detachment solution (Cat. No. 561527) and fixed in 0.5X BD Cytofix™ fixation buffer (Cat. No. 554655). They were then stained according to kit instructions, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri™ C6 software. **Results: A.** Cells were distinguished from debris and gated using light scatter. **B.** The $CD13^-$ gate excludes the 5.0% of cells that still express markers of the starting fibroblast cell population. **C.** Of the gated $CD13^-$ cells, 32.9% expressed the pluripotency markers TRA-1-60 and SSEA-4. Note that cells can also be analyzed live.

## Ordering Information

All kits and their associated software templates are available at **bdbiosciences.com/go/templates**.

| Description | Clone | Quantity | Number of Tests | Cat. No. |
|---|---|---|---|---|
| **BD Stemflow™ Human Pluripotent Stem Cell Analysis and Sorting Kit containing:** | | | | |
| CD13 PerCP-Cy™5.5 | WM15 | 5 µL | 50 tests | **562626** |
| TRA-1-60 PE | TRA-1-60.1 | 5 µL | | |
| SSEA-4 Alexa Fluor® 647 | MC-813-70.1 | 5 µL | | |
| Controls and compensation particles as detailed in technical datasheet | | | | |

## Related Kits

| Description | Cat. No. |
|---|---|
| BD Stemflow™ Human Pluripotent Stem Cell Sorting and Analysis Kit | **560461** |
| BD Stemflow™ Human Pluripotent Stem Cell Transcription Factor Analysis Kit | **560589** |
| BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit | **560477** |
| BD Stemflow™ Human Definitive and Pancreatic Endoderm Analysis Kit | **562496** |
| BD Stemflow™ Human Neural Lineage Analysis Kit | **561526** |

## References


1. 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.

2. 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.





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