

# BD Stemflow Pluripotent Stem Cell Transcription Factor Analysis Kits

## Features

Provides intracellular, multimarker data (expression of Nanog, Oct3/4, and Sox2) on ES and iPS cells

Delivers a streamlined solution for consistent experiments with fluorochrome conjugated antibodies, isotype controls, compensation beads, buffers, protocols, and analysis guidelines

Facilitates compensation and scatter setup and conserves cells with BD CompBead Plus microparticles

Provides flexibility to drop in additional fluorescently conjugated antibodies and to use cell lines expressing GFP and EGFP

The BD Stemflow™ Pluripotent Stem Cell Transcription Factor Analysis Kits are comprehensive research systems for simultaneously measuring expression of the stem cell master regulators—Nanog, Oct3/4, and Sox2—in heterogenous stem cell populations.

The ready-to-use flow cytometry kits, optimized for either mouse or human analysis, maximize reproducibility and improve productivity by integrating pre-conjugated antibodies to markers for pluripotency, compensation beads, staining and fixation buffers, verified protocols, and software analysis guidelines.

An open design provides flexibility to meet your specific research objectives by enabling the easy addition of supplementary fluorochrome-conjugated antibodies and the use of cells expressing green fluorescent protein (GFP) and enhanced green fluorescent protein (EGFP).

## Multicolor Flow Cytometry for In-depth Analysis

Capitalizing on the powerful capabilities of multicolor flow cytometry, the kits allow researchers to perform multiparameter analysis at the single-cell level, enabling deep insight into cell identity and function. Data on the relative expression level of multiple markers can be obtained for individual pluripotent or differentiated cells.

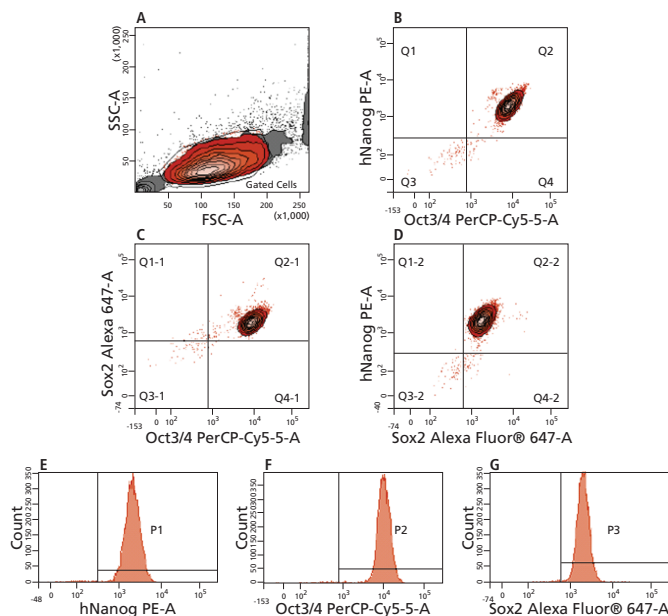
## A Total Solution System to Minimize Variability

Monoclonal antibodies specific to three well known pluripotency markers (Nanog PE, Oct3/4 PerCP-Cy™5.5, and Sox2 Alexa Fluor® 647) and corresponding isotype controls are pre-titrated and pre-conjugated to streamline procedures. BD™ CompBead Plus microparticles are designed to simplify experimental setup by facilitating compensation for multicolor analysis of ES cells and iPS cells. The use of BD CompBead microparticles also eliminates the need to use precious cells as unstained or single-stained cellular controls for compensation.

Fixation and staining buffers and protocols and software analysis protocols standardize procedures to reduce experiment-to-experiment variability.

## Modular and Open to Accommodate Specific Needs

For simple customization and more advanced analysis, the open, modular architecture of the kits allow for the easy addition of supplementary fluorochrome-conjugated monoclonal antibodies against critical cell-surface or intracellular markers. In addition, the kits use a multicolor fluorochrome combination that is compatible with cell lines expressing GFP and EGFP. BD CompBead microparticles can also be used as compensation controls for additional antibody drop-ins to these kits.



**Figure 1.** The pluripotency status of human ES (H9) cells analyzed using the BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit.

Human ES cells were gated after cytometer setup using BD CompBead Plus (Figure 1A). Undifferentiated Nanog<sup>+</sup>Oct3/4<sup>+</sup>, Oct3/4<sup>+</sup>Sox2<sup>+</sup>, and Nanog<sup>+</sup>Sox2<sup>+</sup> human ES cells composed 99%, 99%, and 99% of the cell population, respectively (Figure 1 B,C,D). Histograms displaying cell counts versus the relative expression level of individual markers are also shown (Figure 1 E,F,G).

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## A Resource for Stem Cell Research

With more than 25 years of successful experience in the field, BD Biosciences continues to support innovation in the area of stem cell research. Inspired by in-depth understanding of the complexities of biological experiments, the BD Stemflow Pluripotent Stem Cell Transcription Factor Analysis Kits are designed to make it easier for researchers to obtain accurate results, increase research productivity, and accelerate discoveries.

## Ordering Information

Description	Cat.No.
BD Stemflow™ Human Pluripotent Stem Cell Transcription Factor Analysis Kit (50 tests)	560589
BD Stemflow™ Mouse Pluripotent Stem Cell Transcription Factor Analysis Kit (50 tests)	560585

## Contents

Monoclonal Antibodies
Mouse anti-human Nanog PE or Mouse anti-mouse Nanog PE
Mouse anti-Oct3/4 PerCP-Cy5.5
Mouse anti-Sox2 Alexa Fluor® 647
Isotype Controls
Mouse IgG <sub>1</sub> , κ Isotype Control PE
Mouse IgG <sub>1</sub> , κ Isotype Control PerCP-Cy5.5
Mouse IgG <sub>2b</sub> , κ Isotype Control Alexa Fluor® 647
Other Reagents
BD™ CompBead Plus Anti-Mouse Ig, κ
BD CompBead Plus Negative Control
BD Perm/Wash™ Buffer
BD CytoFix™ Buffer
Protocols and Analysis Guidelines
Protocol for cell staining
Protocol for analysis, including creating BD FACSDiva™ templates

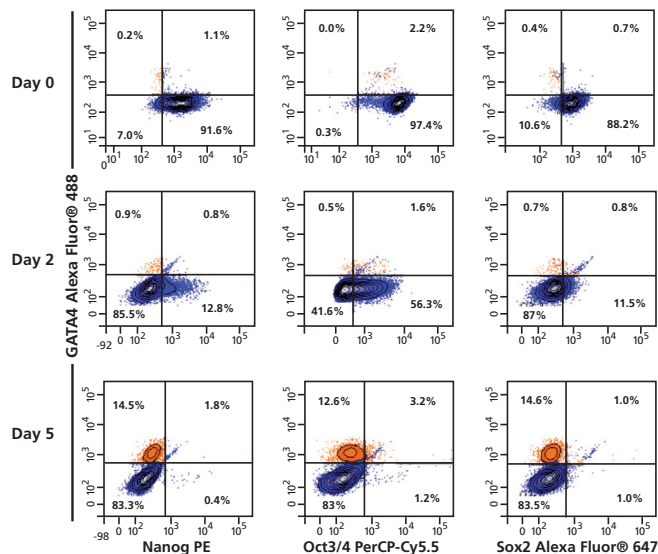


Figure 2. Differentiation of mESCs to mesoderm monitored by flow cytometry.

Mouse ES cells (ESE14TG2a) were treated with 10  $\mu$ M retinoic acid for 5 days. Cells were harvested on day 0, day 2, and day 5. Cells were analyzed for pluripotency and differentiation status using the BD Stemflow Mouse Pluripotent Stem Cell Transcription Factor Analysis Kit. In addition to the pluripotency markers Nanog, Oct3/4, and Sox2, the transcription factor GATA4 was simultaneously analyzed using a GATA4 Alexa Fluor® 488 (Cat. No. 560330) drop-in antibody. After a 5-day treatment with retinoic acid, the percentage of cells expressing Nanog, Oct3/4, and Sox2 markedly decreases while the percentage of cells expressing GATA4 increases.

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