BD FACS™ Pre-Sort Buffer

Features

- Formulated to minimize cell clumping during the preparation, staining, washing, and sorting of cells by fluorescence-activated cell sorting (FACS)
- Optically clear buffer with no phenol red to reduce background
- Provides ideal pH regulation to maintain cell health during cell preparation and sorting
- Compatible with multiple cell types including adherent and sensitive cells

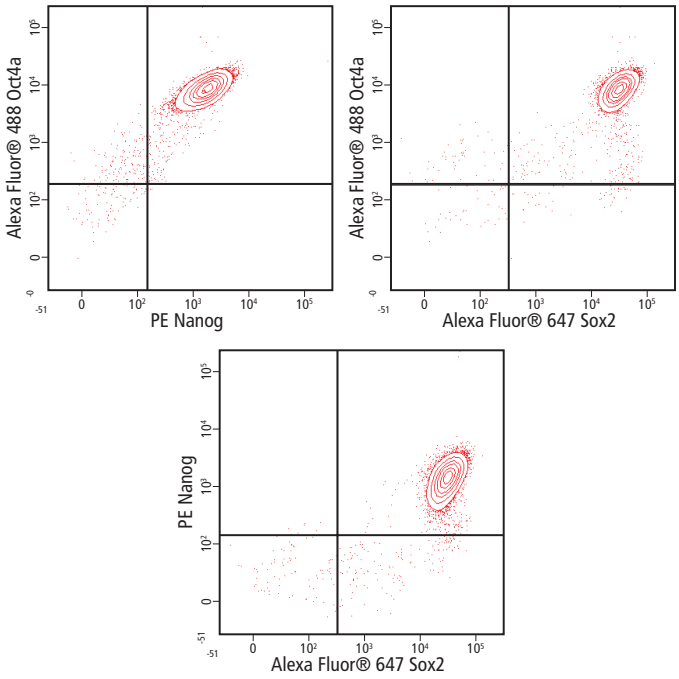

Figure 1. Post-sort analysis of human embryonic stem cells (hESCs)

H9 hESCs (WiCell, Madison, WI) were dissociated using BD Accutase™ Cell Detachment Solution (Cat. No. 561527). Cells were washed, stained with BD Pharmingen™ Alexa Fluor® 647 Mouse Anti-Human TRA-1-81 (Cat. No. 560793) and BD Horizon™ BV421 Rat Anti-SSEA-3 (Cat No. 562706), and maintained in BD FACS Pre-Sort Buffer during sort. Cells were sorted on a BD FACSAria™ III flow cytometer system. The sorted cells were then plated in 6-well tissue culture dishes at approximately 2 million cells per well with BD™ ROCK Inhibitor (Y-27632) (Cat. No. 562822) for 24 hours and cultured for 4 days. Cells were then analyzed for pluripotency. The cultures were dissociated using BD Accutase Cell Detachment Solution, fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were then stained with BD Pharmingen™ Alexa Fluor® 488 Mouse Anti-Oct3/4 (Human Isoform A) (Cat. No. 561628), BD Pharmingen™ PE Mouse Anti-Human Nanog (Cat. No. 560483), and BD Pharmingen™ Alexa Fluor® 647 Mouse Anti-Sox2 (Cat. No. 562139). Cells were then washed and analyzed using a BD LSRFortessa™ X-20 cytometry system. Sorted cells retained a pluripotent phenotype post-sort as shown by the expression of Oct4, Sox2, and Nanog.

BD FACS™ Pre-Sort Buffer is a convenient, ready-to-use buffer for cell sorting applications. It can be utilized in multiple steps of the cell sorting process from cell washing following dissociation (including neutralizing enzymes like trypsin in lieu of media), to cell staining and washing, to running the cells on a cell sorter. Sorted cells can subsequently be collected into a collection buffer of choice and then used in downstream applications.

Optimized components for cell sorting applications

The BD FACS Pre-Sort Buffer is phosphate-based and has minimal Ca^{2+} and Mg^{2+} to minimize cell aggregation. Ca^{2+} and Mg^{2+} are required for many cell adhesion molecules to function properly. The BD FACS Pre-Sort Buffer does not contain EDTA, which can have deleterious effects on cells. It is optically clear to allow for the highest resolution possible when using fluorescently labeled cells or cells stained with fluorochrome-conjugated antibodies, and has been formulated to facilitate consistent droplet formation and stream stability on a cell sorter. Having a stable stream and consistent drop formulation is critical for sorting efficiency to ensure optimal purity and yield of the sorted target population. Additionally, the BD FACS Pre-Sort Buffer contains serum-based protein to help maintain cells in a viable state during cell sorting applications. Each lot of BD FACS Pre-Sort Buffer is endotoxin-tested to be less than 0.96 EU/mL.

Tested and formulated to be compatible with multiple cell types

The BD FACS Pre-Sort Buffer has been tested in multiple cell types, sources, and species from bone marrow to peripheral blood to cultured cells, including sensitive and adherent cells such as human pluripotent stem cells and their derivatives. **Table 1** provides a sampling of cell types prepared, stained, and maintained in the BD FACS Pre-Sort Buffer during the cell sort, as well as the assays performed post-sort to assess the sorted cell populations. To sort some cell types, researchers can choose to add DNase II (to avoid clumpiness from the presence of soluble DNA if excessive cell death is expected in the prep) and/or penicillin and streptomycin to the BD FACS Pre-Sort Buffer. The BD FACS Pre-Sort Buffer is also compatible with commonly used viability dyes such as 7-AAD.

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Table 1. Cells sorted using BD FACS Pre-Sort Buffer

Cell Type	Post-Sort Assay
T Cell[a]	Stimulation potential, cytokine production, viability
T Regulatory Cell[b]	Expansion, viability
H9 Human Embryonic Stem Cell (hESC)[a]	Immunophenotype, morphology, viability
H9 hESC-Derived Neural Stem Cell[b]	Differentiation potential, immunophenotype, viability
H9 hESC-Derived Neuron[b]	Immunophenotype, morphology, viability
Human Bone Marrow[b]	Colony forming unit assay, viability
Mouse Bone Marrow[b]	Colony forming unit assay, viability

[a]With and without DNAse II. Penicillin/streptomycin added.

[b]With DNAse II. Penicillin/streptomycin were added to BD FACS Pre-Sort Buffer.

All cells were sorted on a BD FACSAria™ II, III, or Fusion flow cytometry system.

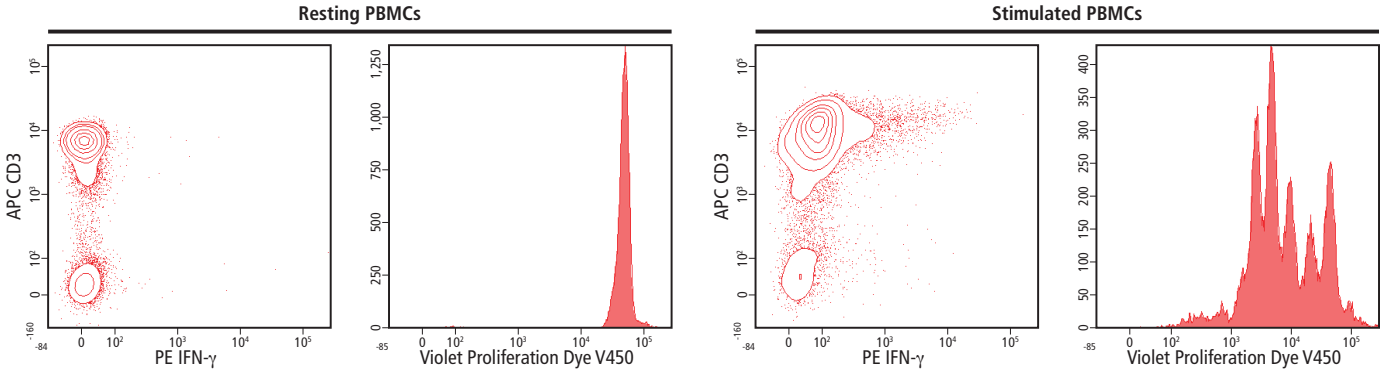

Figure 2. Activation of sorted lymphocytes

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient separation (Ficoll-Paque™, GE Healthcare). PBMCs were then washed and resuspended in BD FACS Pre-Sort Buffer prior to cell sorting. Lymphocytes were sorted based on light scatter properties and were allowed to recover in culture for 24 hours in growth medium. At the end of the recovery period, cells were stained with BD Horizon™ Violet Proliferation Dye 450 (Cat. No. 562158) and stimulated with Dynabeads® Human T-Activator CD3/CD28, according to the manufacturer's instructions (Life Technologies). After three days of stimulation, cells were treated with BD GolgiStop™ Protein Transporter Inhibitor (Cat. No. 554724) for 6 hours, then fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (Cat. No. 554714), and stained with BD Pharmingen™ APC Mouse Anti-Human CD3 (Cat. No. 555335) and BD Pharmingen™ PE Mouse Anti-Human IFN-γ (Cat. No. 559326). Flow cytometry was performed on a BD LSRFortessa flow cytometry system. Sorted cells not activated with CD3/CD28 beads were used as a negative control. As expected, no expression of IFN-γ or proliferation was observed within the lymphocyte population. After three days of exposure to CD3/CD28 beads, stimulated $CD3^+$ lymphocytes expressed IFN-γ and proliferated, as shown by the serial decrease in VPD450 intensity.

Ordering Information

Description	Size	Cat.No.
BD FACS Pre-Sort Buffer	250 mL	**563503**

23-17142-00

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