Technical Data Sheet

Human Definitive and Pancreatic Endoderm Analysis Kit

Product Information

Material Number: **562496**
Size: 25 Tests
Reactivity: QC Testing: Human

Description

The BD Stemflow™ Human Definitive and Pancreatic Endoderm Analysis Kit contains a combination of mouse monoclonal antibody conjugates for the analysis of definitive and pancreatic endodermal differentiation cultures. The antibody specificities that are provided in this kit and the cell populations they can identify are listed in the table below. The antibody components of this kit can be used individually or in combinations of up to four. Additional antibody formats that can enable more complex panels are available at www.bdbiosciences.com. The kit also includes BD Cytofix™ Fixation Buffer and BD Phosflow™ Perm Buffer III.

Kit Components

Component	Description	Size	Vol. Per Test	Storage Buffer
51-9008006	Alexa Fluor® 488 Mouse Anti-Human Pax-6	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9008007	PE Mouse Anti-PDX-1	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9008008	PE Mouse Anti-Human FoxA2	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9007227	PerCP-Cy™5.5 Mouse Anti-Sox2	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9008009	PerCP-Cy™5.5 Mouse Anti-Human Sox17	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9008010	Alexa Fluor® 647 Mouse Anti-Human Nanog	25 Test	5 µl	Aqueous buffered solution containing BSA and ≤0.09% sodium azide
51-9006607	Perm Buffer III	50 ml		
51-9006276	Fixation Buffer	50 ml		

Specificity	Clone	Cell Population Identified
Nanog	N31-335	Pluripotent stem cells
Sox2	O30-678	Pluripotent stem cells, ectodermal contaminants
Sox17	P7-969	Definite endoderm
FoxA2	N17-280	Definitive endoderm
Pax6	O30-1330	Pancreatic endoderm, ectodermal contaminants
Pdx1	658A5	Pancreatic endoderm

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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Recommended Assay Procedure:

1. Detach cells of interest from the culture dish. Investigators are encouraged to detach cells at 37°C using Accutase™ Cell Detachment Solution (Cat. No. 561527). Mild to moderate triturating of the cell suspension is recommended to achieve a single cell suspension.

2. Wash the cells in 1X PBS. If clumps are present, cells can be filtered using a 70 mm Falcon® cell strainer (Corning Cat. No. 352350).

3. Resuspend the cells in 0.5X BD Cytofix™ Fixation Buffer (Cat. No. 554655) diluted in 1X PBS and incubate at 37°C for 10 minutes. Recommended volumes for fixing cells is 1 ml of fixation solution per 10 million cells with a minimum volume of 200 µl.

4. Wash the cells twice with 1X PBS. If investigators wish to continue at a later time, cells may be frozen and stored in a solution of 10% DMSO + 90% FBS at -80°C using standard methods for the cryopreservation of live cells.

5. Vortex to loosen the cell pellet and then permeabilize the cells by slowly adding ice cold BD Phosflow™ Perm Buffer III (Cat. No. 558050) while vortexing. Recommended volumes for permeabilizing cells is 1 ml of BD Phosflow™ Perm Buffer III per 10 million cells with a minimum volume of 1 ml.

6. Incubate on ice for 30 minutes, or alternatively, the cells can be stored in BD Phosflow Perm Buffer III for up to 6 months at -20°C.

7. Wash the cells twice with BD Pharmingen™ Stain Buffer (FBS) (Cat. No.554656) and resuspend at 5 - 10 million cells/ml in BD Pharmingen™ Stain Buffer (FBS). Alternatively, 1X Washing/Staining Solution (1 x PBS,1% FCS, and 0.09% sodium azide) may be used.

8. Add 100 µl cells into appropriately labeled 12 x 75mm polystyrene tubes.

9. Add panel of antibody conjugates that you wish to use (5 µL/test of each antibody conjugate) and incubate in the dark for 30 minutes.

10. Wash twice with 2 ml BD Pharmingen™ Stain Buffer (FBS).

11. Resuspend sample in appropriate volume (350-400 µL) of BD Pharmingen™ Stain Buffer (FBS) to run on a flow cytometer.

Kit Considerations:

Choosing a Cell Detachment Enzyme: Investigators are encouraged to use Accutase™ Cell Detachment Solution (Cat. No. 561527), as cell death with this detachment method has been observed to be minimal.

Fixation and Permeabilization Methods: The components of this kit are all compatible with each other. However if additional specificities are included, those antibody conjugates must be verified to work with the fix/perm buffers included in this kit.

Addition of Cell Surface Markers: CD184 PE (Cat. No. 555974), CD184 APC (Cat. No. 555976), and CD184 BV421 (Cat. No. 562448) have been used successfully with components of this kit. However, as non-specific background staining is seen on pluripotent stem cells that have been fixed then stained with CD184, it is recommended that cells are detached, stained live with CD184, fixed as recommended above, permeabilized with 0.1% Triton™ X-100 (Perm III can negatively impact CD184 positive stain), and then stained for intracellular markers.

Instrument Set Up: BD™ CompBead Plus particles (Cat. No. 560497) may be beneficial in setting up the experiment on a flow cytometer. Investigators are encouraged to set up PMT voltages with the BD™ CompBead Plus particles and then checking to ensure the voltage settings are appropriate by running a stained sample of cells before running the entire experiment.

Data Analysis: A cluster based gating approach is recommended when analyzing data.

Cell Populations: This kit has been developed for use on endodermal cell populations that have been derived from human pluripotent stem cells using the methods described in the figure legends below. This kit has not been tested on other cell types, including primary cells, and results may differ when examining cell types of a different origin (e.g higher background staining). In these instances, investigators may find it helpful to use isotype controls to distinguish non-specific background staining from specific antibody staining.

Warnings & Precautions

Danger: BD Phosflow™ Perm Buffer III (component 51-9006607) contains 87.68% methanol (w/w).

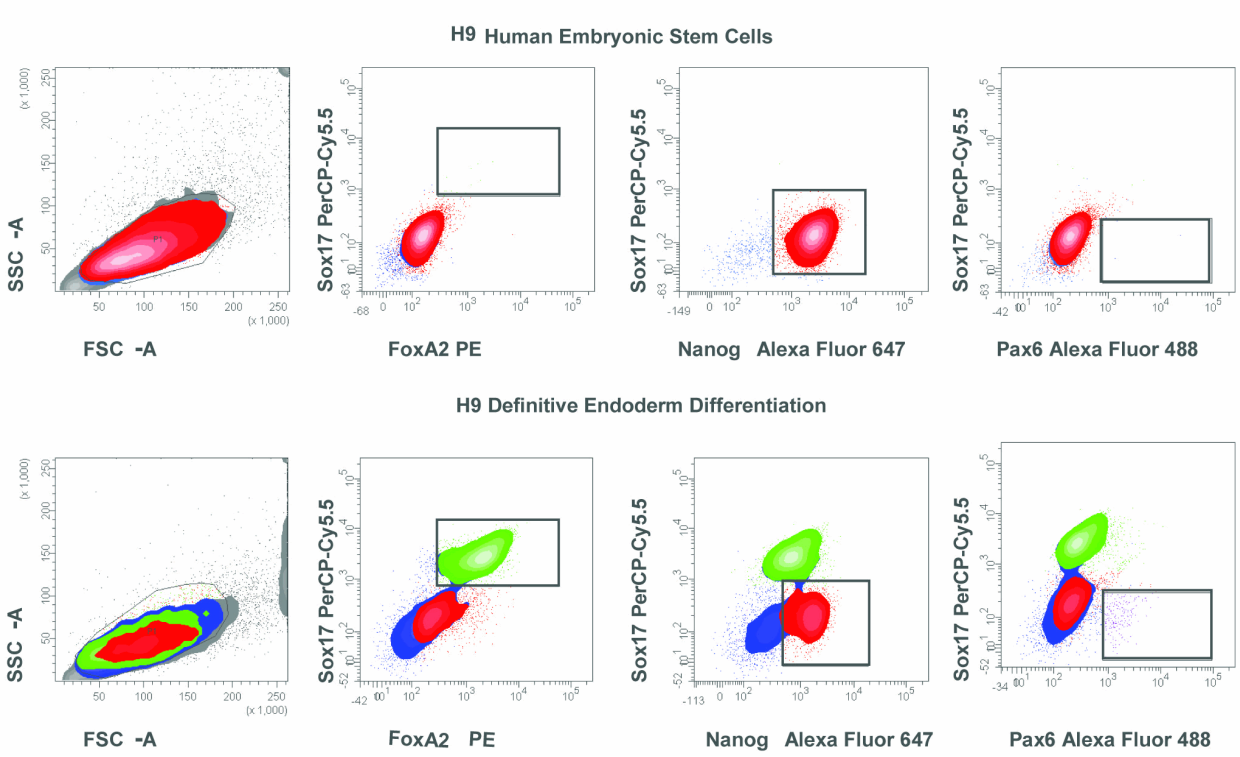
Hazard statements
Highly flammable liquid and vapor.
Toxic if swallowed, in contact with skin or if inhaled.
Causes damage to the central nervous system. Route of exposure: Oral.

Precautionary statements
Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
Wear protective clothing / eye protection.
Wear protective gloves.
Do not breathe mist/vapours/spray.
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Danger: BD Cytofix™ Fixation Buffer (component 51-9006276) contains 4.2% formaldehyde (w/w).

Hazard statements
Harmful if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.
May cause respiratory irritation.

Precautionary statements
Wear protective clothing / eye protection.
Wear protective gloves.
Do not breathe mist/vapours/spray.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If skin irritation or rash occurs: Get medical advice/attention.



Definitive Endoderm differentiation of H9 hESC: *H9 human embryonic stem cells (hESCs) (WiCell, Madison, WI) (**upper panels**) and differentiated cells (**lower panels**) were analyzed by flow cytometry using components of the BD Stemflow™ Human Definitive and Pancreatic Endoderm Analysis Kit. H9 hESCs were cultured on irradiated mouse embryonic fibroblasts in hESC medium (DMEM:F12, 20% Knockout Serum Replacement (KOSR), non-essential amino acids, 20 mM glutamine) (Life Technologies), 1X penicillin/streptomycin (Lonza), bFGF (Cat. No. 354060). For definitive endoderm differentiation, hESCs were differentiated for three days in 0.5% FBS, 20mM glutamine, RPMI (Life Technologies) and 100ng/ml Activin A (R&D Systems) (D'Amour et al., 2005). Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

Suggested Companion Products

Catalog Number	Name	Size	Clone
561527	Accutase™ Cell Detachment Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
560497	Anti-Mouse Ig, κ/Negative Control (BSA) Compensation Plus (7.5 µm) Particles Set	6 mL	(none)
558055	Alexa Fluor®488 Mouse IgG2a, κ Isotype control	50 Tests	MOPC-173
555974	PE Mouse Anti-Human CD184	100 Tests	12G5
553457	PE Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178
555976	APC Mouse Anti-Human CD184	100 Tests	12G5
550882	APC Mouse IgG2a κ Isotype Control	0.1 mg	G155-178
562448	BV421 Mouse Anti-Human CD184	50 Tests	12G5
562439	BV421 Mouse IgG2a, k Isotype Control	50 µg	G155-178
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
557732	Alexa Fluor® 647 Mouse IgG1 κ Isotype Control	100 Tests	MOPC-21

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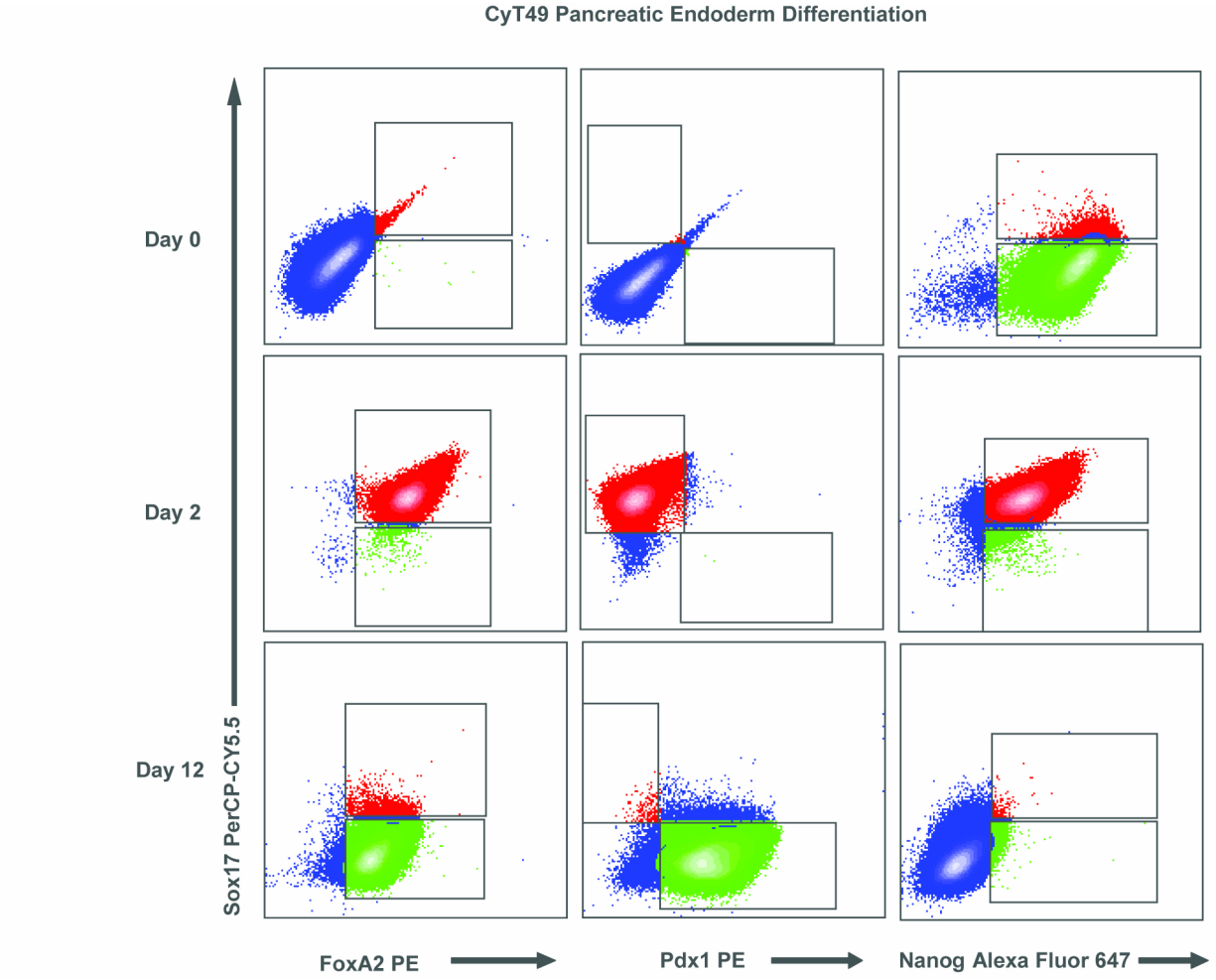

***Pancreatic endoderm differentiation of CyT49 hESCs**: CyT49 Human embryonic stem cells (hESCs) and differentiated cells were analyzed by flow cytometry using components of the BD Stemflow™ Human Definitive and Pancreatic Endoderm Analysis Kit. CyT49 hESCs were cultured and differentiated as previously described (D'Amour et al., 2006; Kroon et al., 2008, and Kelley et al., 2011). Flow cytometry was performed on a BD™ LSR II flow cytometry system. (Data provided by ViaCyte, Inc.)*

Product Notices

1. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
2. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
3. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
4. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
9. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
10. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.

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11. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
12. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
13. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
14. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
15. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol.* 2005; 23(12):1534-1541. (Methodology: Cell differentiation)

D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol.* 2006; 24(12):1481-1483. (Methodology: Cell differentiation)

Kelly OG, Chan MY, Martinson LA, et al. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat Biotechnol.* 2011; 29(8):750-756. (Methodology: Cell separation)

Kroon E, Martinson LA, Kadoya K, Bang AG, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol.* 2008; 26(4):443-452. (Biology: Cell differentiation)

Wang P, Rodriguez RT, Wang J, Ghodasara A, Kim SK. Targeting SOX17 in Human Embryonic Stem Cells Creates Unique Strategies for Isolating and Analyzing Developing Endoderm. *Cell Stem Cell.* 2011; 8:335-346. (Biology)

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