Technical Data Sheet

HumanTh1/Th2/Th17 Phenotyping Kit

Product Information

Material Number: **560751**
Size: 50 tests

Description

Components:

51-9006615	**Human Th1/Th2/Th17 Phenotyping Cocktail**		**1.0 ml**
	Containing the following:		
	Human CD4 PERCP-CY5.5	**Clone: SK3**	
	Human IL-17A PE	**Clone: N49-653**	
	Human IFN-GMA FITC	**Clone: B27**	
	Human IL-4 APC	**Clone: MP4-25D2**	
51-9006613	**BD Cytofix™ Fixation Buffer**		**100 ml**
51-2091KE	**BD Perm/Wash™ Buffer**		**25 ml**
51-2092KZ	**BD GolgiStop™ Protein Transport Inhibitor (containing monensin)**		**0.7 ml**

The peripheral CD4+ T cell pool includes multiple effector and memory T cell subsets that arise through antigen-driven expansion and differentiation of naïve T cells. The early response of naïve CD4+ T cells to antigenic stimulation is characterized by high level proliferation and a limited cytokine repertoire. Further differentiation yields cells with a more diverse potential for cytokine expression. Depending upon the balance of local cytokines, costimulatory molecules, antigen levels, and genetic factors, Type-1 T helper (Th1), Th2, and Th17 effector and/or memory cells are generated by immune responses.

Functionally-polarized CD4+ T cell subsets have been identified based on their distinctive patterns of cytokine secretion. As a signature cytokine, Th1 cells selectively produce large amounts of interferon-gamma (IFN-γ). Th2 cells selectively produce IL-4, and Th17 express high levels of IL-17A. Through secretion of IFN-γ and other effector molecules, Th1 cells activate macrophages, natural killer (NK) cells, and CD8+ T cells and are responsible for cell-mediated immunity. Th1 cells provide protection against intracellular bacteria, fungi, protozoa and viruses and are involved in some autoimmune responses. IL-4 produced by Th2 cells is particularly strong in driving B cells to generate IgE-secreting cells. IgE plays a role in basophil/mast cell mediated immune reactions. Th2 cells mediate protection against extracellular parasites but may also cause harmful allergic responsiveness to develop. Through the secretion of IL-17A and other factors, Th17 cells recruit and activate neutrophils and mediate immune responses against extracellular bacteria and fungi. Th17 cells are also implicated in autoimmune responses. In addition to these types of T helper cells, Th0- (IL-4 and IFN-γ) and Th17/Th1-like (IL-17A/IFN-γ) cells that coexpress signature cytokines have been described.

The Th1/Th2/Th17 paradigm provides a useful model system for investigating the cellular and molecular mechanisms that mediate protective as well as harmful immune responses. The BD Human Th1/Th2/Th17 Phenotyping Kit provides an easy-to-use four-color cocktail of fluorescent antibodies-specific for human CD4, IFN-γ (for Th1), IL-4 (for Th2) and IL-17A (for Th17)-that will enable researchers to identify and characterize the nature of these T helper cell types by multicolor flow cytometric analysis. The kit can be used to successfully analyze ex vivo lymphoid cell samples (eg, for the types of in vivo-generated human peripheral blood T helper cells) or to monitor T helper cell differentiation by cells cultured within various experimental model systems.

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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.

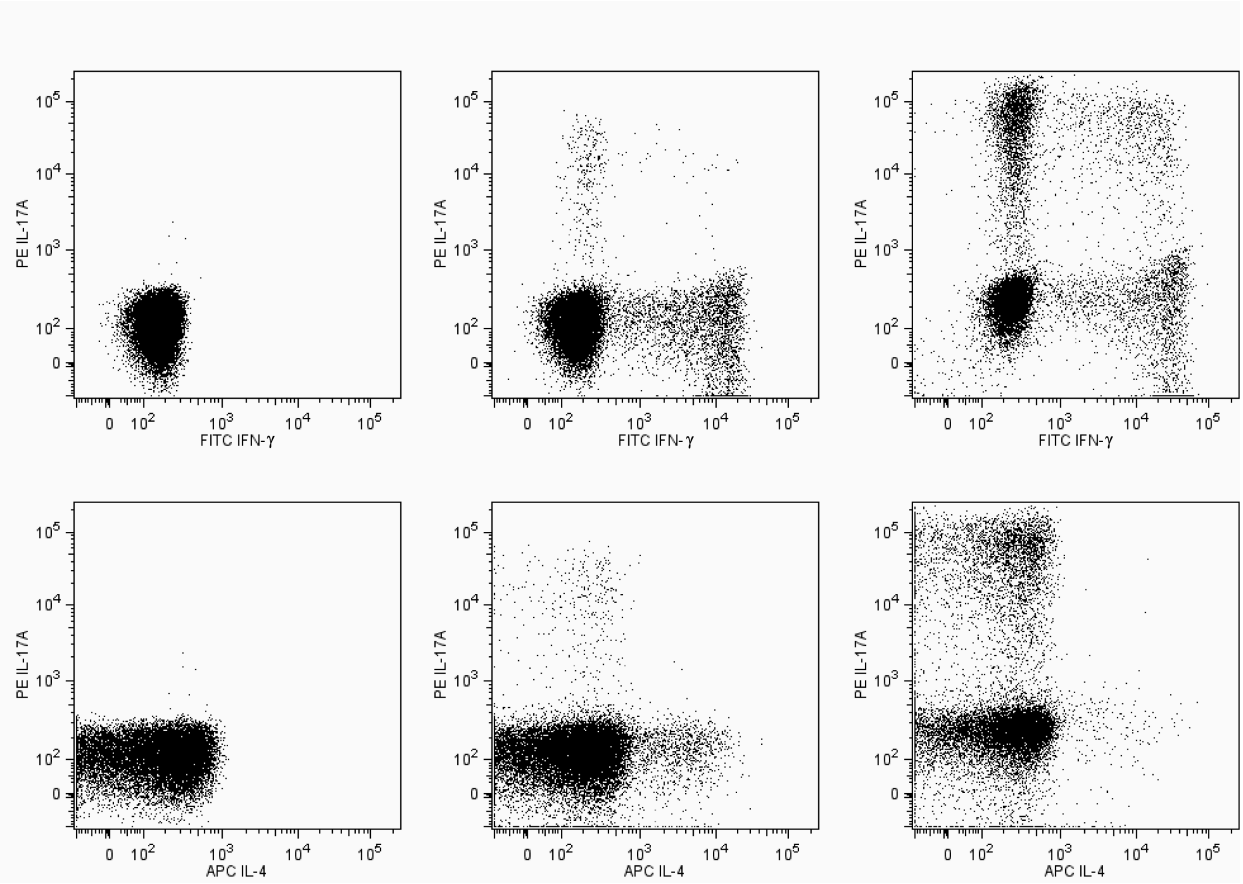
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Irritating to eyes and skin. Do not breathe vapor. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Warnings and Precautions:

R11	Highly flammable.
R40	Limited evidence of a carcinogenic effect.
R43	May cause sensitization by skin contact.
S2	Keep out of reach of children
S7/9	Keep container tightly closed and in a well-ventilated place.
S13	Keep away from food, drink and animal feedingstuffs.
S16	Keep away from sources of ignition - No smoking.
S23	Do not breathe gas/fumes/vapour/spray
S25	Avoid contact with eyes.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.
S52	Not recommended for interior use on large surface areas.
S60	This material and its container must be disposed of as hazardous waste.



Flow cytometric analysis of human Th1/Th2/Th17 phenotyping kit. *The staining pattern of IFN-γ, IL-17A and IL-4 on resting PBMCs (left column), PMA/Ionomycin stimulated PBMCs (middle column) and polarized Th17 cells (right column) are shown. The top three panels show the staining of IL-17A vs. IFN-γ and the bottom three panels show the staining of IL-17A vs. IL-4. Dot plot analyses are derived from gated CD4+ cell populations. Flow cytometry was performed on a BD™ LSR II System.*

Application Notes

Application

Flow cytometry	Tested During Development

Recommended Assay Procedure:

General Protocol for human Th1/Th2/Th17 phenotyping kit

A. Stimulation of the Cells

Various *in vitro* methods have been reported for polarization or stimulation of T helper cells subsets of which PMA (Phorbol ester) plus Ionomycin (Calcium Ionophore) has been particularly useful for quickly inducing and characterizing polyclonal cytokine-producing cells. For this kit we recommend the stimulation of normal PBMCs at a concentration of 1-10 million cells per ml in media for 5 hours with PMA/Ionomycin (at 50ng/ml and 1µg/ml respectively) in the presence of BD GolgiStop™ Protein Transport Inhibitor (provided in the kit or Cat #554724). Add 4 µl of BD GolgiStop™ for every 6 ml of cell culture and mix thoroughly. It is recommended that BD GolgiStop™ not be kept in cell culture for longer than 12 hours.

Note: Kinetic studies need to be performed to determine the optimal incubation time for each experimental system. Depending on the donor, frequencies of cytokine producing cells derived from activation of PBMC can vary widely for a specific cytokine. In particular, the number of IL-17 producing cells can be very low or even negligible on PMA/Ionomycin stimulated PBMC. In these cases, Th17 polarization cultures should be considered. For specifics on polarization of Th17 cells please refer to the references.

B. Staining of the Cells

1. Harvest of the Cells

Collect cells from *in vitro* stimulatory cultures treated with a protein transport inhibitor. Spin down cells at 250 x g for 10 minutes at room temperature (RT) and wash two times with stain buffer (FBS) (Cat# 554656). Count cells and transfer approximately 1 million cells to each flow test tube (Cat# 352008) for immunofluorescent staining. Cells should be protected from light throughout the staining procedure and storage.

2. Fixing the Cells

a. Spin down cells at 250 x g for 10 minutes at RT and thoroughly suspend cells with 1ml of cold BD Cytofix™ buffer (provided in the kit or Cat# 554655) and incubate for 10-20 minutes at RT.

Note: Cell aggregation can be avoided by vortexing prior to the addition of the fixative.

b. Spin down cells at 250 x g for 10 minutes at RT.

*Note: After fixation, the cell pellet after centrifugation is loose and care should be taken when aspirating the wash buffer from the tubes. Do not aspirate **ALL** of the buffer but leave 50-150 µl of solution in the tubes to avoid cell loss for all subsequent wash steps below.*

c. Wash cells twice at RT in stain buffer (FBS) and spin down the cells at 250 x g for 10 minutes at RT.

Note: Cells can be stored in stain buffer at 4°C for up to 72 hours or in 90% FCS/10% DMSO at -80°C for up to six months (for samples that need to be stored longer than six months, we recommend performing stability studies) .

3. Permeabilizing the Fixed Cells

a. For cells kept at 4°C, spin down cells at 250 x g for 10 minutes at RT and remove stain buffer.

b. For cells stored at -80°C thaw and wash twice with stain buffer (FBS) to remove DMSO.

c. Dilute 10x BD Perm/Wash™ buffer (provided in the kit or Cat# 554723) in distilled water to make a 1x solution prior to use.

d. Suspend cells in 1ml of 1x BD Perm/Wash™ buffer and incubate at RT for 15 minutes.

e. Spin down cells at 250 x g for 10 minutes at RT and remove supernatant.

4. Staining with the Cocktail

a. Thoroughly suspend fixed/permeabilized cells in each tube in 50 µl of BD Perm/Wash™ buffer and add 20 µl/tube of cocktail or appropriate negative control. Incubate at RT for 30 minutes in the dark. Cells should be protected from light throughout the staining procedure and storage.

b. Optional: Staining of Additional Cell Surface Antigens

Note: If instrument allows, optional multicolor staining of different cell surface antigens can be done at this time. Example: PE-Cy™7 CD3 (Clone SK7, Cat# 557851), V450 CD8 (CloneRPA-T8, Cat# 560347) and V450 CD45RA (Clone HI100, Cat# 560362) etc. For cell surface staining after fixation/permeabilization, suitable antibody clones that recognize denatured epitopes need to be identified.

Note: For antibodies that do not recognize fixed/denatured cell surface markers, it is recommended that staining be done on live cells PRIOR to fixation/permeabilization.

c. Wash cells twice with 1ml of 1× BD Perm/Wash™ buffer at RT and suspend in Stain Buffer (FBS) prior to flow-cytometric analysis.

C. Flow Cytometric Analysis

Set PMT voltage and compensation using unstained cells and appropriate cell surface markers or use BD™ Compensation beads (Cat # 552844; 560499) as per the recommended protocol.

Note: It has been reported that CD4 expression on T cells is decreased after cell activation.

*Note: **Acquire at least 20,000 to 30,000 CD4 positive lymphocytes.** Depending on the donor, frequencies of cytokine producing cells derived from activation of human PBMCs can vary widely for a particular cytokine. In order to make statistically significant frequency measurements, sufficiently large sample sizes should be acquired during flow cytometric analysis. Bivariate dot plots or probability contour plots can be generated upon data reanalysis to display the frequencies of and patterns by which*

individual cells co-express certain levels of cell surface antigen and intracellular cytokine proteins.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
552843	Anti-Mouse Ig, κ/Negative Control (FBS) Compensation Particles Set	6.0 ml	187.1
557851	PE-Cy™7 Mouse Anti-Human CD3	100 tests	SK7
560347	V450 Mouse Anti-Human CD8	120 tests	RPA-T8
560362	V450 Mouse Anti-Human CD45RA	120 tests	HI100
560608	PE-Cy™7 Mouse Anti-Human CD45RO	50 tests	UCHL1
552844	Anti-Rat Ig, κ/Negative Control (FBS) Compensation Particles Set	6.0 ml	G16-510E3
560802	Three-color Fluorescent Ig Isotype Cocktail	20 tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
6. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
7. 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.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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References

Annunziato F, Cosmi L, Liotta F, et al. Human Th17 cells: are they different from murine Th17 cells?. *Eur J Immunol*. 2009; 39:637-640. (Biology)
Annunziato F, Cosmi L, Santarlasci V, et al. Phenotypic and functional features of human Th17 cells. *J Exp Med*. 2007; 204:1849-1861. (Biology)
Romagnani S. Human TH1 and TH2 subsets: doubt no more. *Immunol Today*. 1991; 12:256-257. (Biology)
Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood*. 2008; 112(5):1557-1569. (Biology)