BD Pharmingen™ Transcription Factor Phospho Buffer Set

Features

- Optimized fixation and permeabilization buffer that combines nuclear transcription factor (eg, FoxP3, and T-bet) staining with phosphorylated signaling protein detection
- Sensitive, reproducible, and flexible detection of cellular responses in human and mouse models in freshly isolated cells, thawed cryopreserved samples, or fixed-stored-thawed cryopreserved samples
- Suitable for high-throughput 96-well format and cell barcoding protocols for precise results in large studies

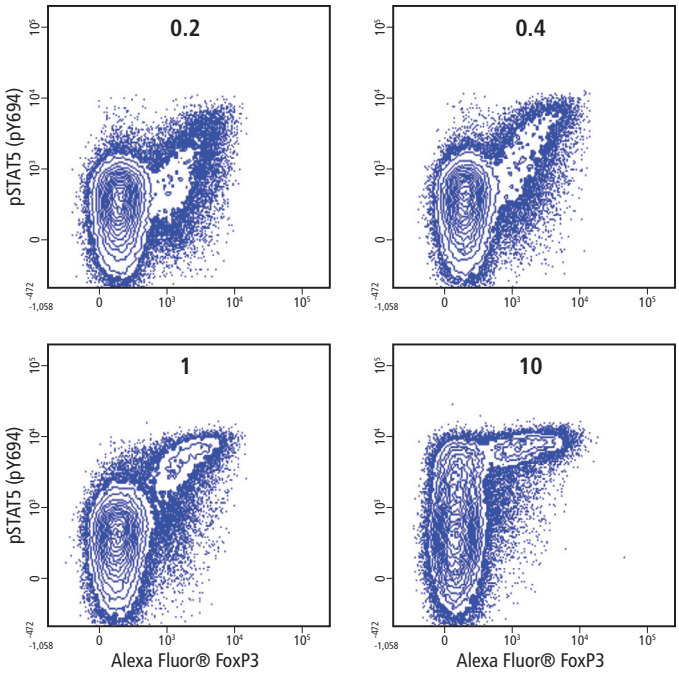

Figure 1. FoxP3 and pSTAT5 (pY694) expression and low concentration IL-2 signaling responses in cryopreserved CD4 T cells

Frozen human PBMC samples were stimulated with 0.2, 0.4, 1 and 10 U/mL (or 12, 24, 61, 611 pg/mL) human IL-2 recombinant protein, fixed, and permeabilized using BD Pharmingen Transcription Factor Phospho Buffer Set (TFP Buffer) and protocol. Cells were labeled with surface and intracellular antibodies after treatment with BD Phosflow™ Perm Buffer III. Phenotyping of Tregs was determined using anti-human CD45RA, CD4, and CD25 antibodies.

Data courtesy of Yang, Hsiu-Mien, King's College London, UK

Combines the power of BD Pharmingen Transcription Factor Buffer with phosphoprotein detection

BD Pharmingen™ Transcription Factor Phospho Buffer Set is a new formulation of a mild fixation and permeabilization buffer sequentially paired with harsh alcohol (BD Phosflow™ Perm Buffer III) and a verified methodology. The buffer system is designed for the simultaneous detection of surface and intracellular antigens; particularly for the detection by flow cytometry of protein phosphorylation in response to stimuli, and the nuclear transcription factors FoxP3 and T-bet in mouse and human leukocytes. The buffer system is compatible with most existing surface staining reagents and tailored to BD Phosflow™ pSTAT antibodies. The buffer has been successfully used to assess signaling responses by leucocyte subsets to cytokines.

Reliably determines phenotype and cell-signaling function by flow cytometry

Protective immunity depends on coordinated responses between regulatory and conventional T-cell subsets. Assessing signaling responses within T-cell subsets defined by the expression of specific transcription factors (TF) may provide insight into immune regulation in healthy and disease states. The BD Pharmingen Transcription Factor Phospho Buffer supports higher intracellular resolution and desirable sample stabilities, allowing detection of proteins of interest. This enables robust simultaneous detection of nuclear TF expression and protein phosphorylation (eg, pSTAT5) in many sample types.

Supports high-throughput testing and the use of precious cryopreserved samples

Concurrent FoxP3 and phospho-STAT expression by flow cytometry presents challenges for obtaining reproducible results, particularly when using frozen samples. Cryopreservation and cell function methods are also of significant practical importance, especially for labile FoxP3+ regulatory T-cell phenotypes, and when cell recoveries are essential for detection of small cell populations. Our optimized protocol allows for highly reproducible results in frozen samples. When using our single-cell assay method to assess signaling responses to cytokines within T-cell subsets, and defined by TF, we can now offer insights into immune regulation on banked samples; human and mouse tissues, and in fresh, fixed, and frozen human PBMC (Figure 1). Options for 96-well sample handling, barcoding, and long term storage additionally allows for a robust study design.

Verified cell signaling in different stimulation conditions

Our studies show successful application using IL-2, IFN-α, IL-6, IFN-γ, IL-4, IL-7, IL-15, and anti-CD3/CD28 for cell stimulations prior to using BD Pharmingen™ Transcription Factor Phospho Buffer Set for the robust detection of pSTAT1, pSTAT3, pSTAT4, and pSTAT5 and other phosphoantigens (Figure 2). In addition, the buffer is compatible with a wide range of reagents (Table 1).

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

BD Pharmingen™ Transcription Factor Phospho Buffer Set

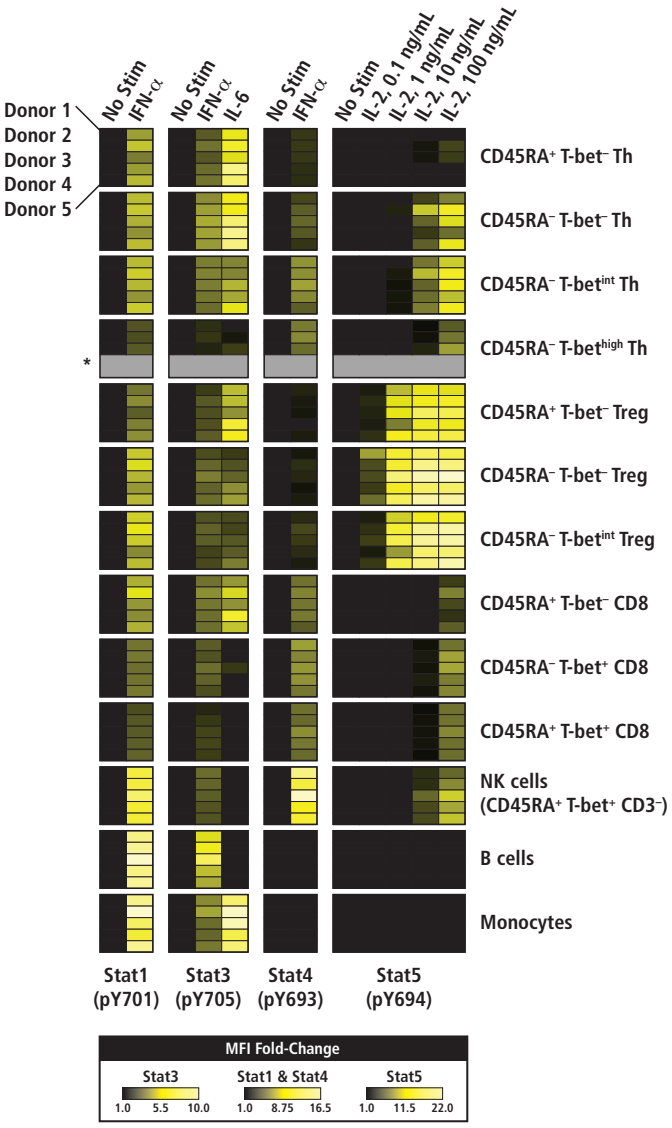

Figure 2. Population-specific induction levels of phospho-STAT single cell signaling in naïve and memory T cells and other leucocyte subsets

Five different human donors (PBMC samples) were stimulated with cytokines, fixed, permeabilized, and stained using TFP Buffer and protocol. Cells were labeled with surface and intracellular antibodies after treatment with BD Phosflow Perm Buffer III. Cell phenotype was determined using anti-human CD3, CD4, CD20, CD25, CD45RA, T-bet, and FoxP3 antibodies. Data was generated using Cytobank™ software.

**Not detected, < 50 events acquired.*

Description	Clone	Reactivity	Formats Tested	Cat. No.
CD3	SK7	Hu	PE	340662
CD3	UCHT1	Hu	PerCP-Cy5.5, BD Horizon™ BV421 and V450	560835, 562427, 560365
CD4	SK3	Hu	BV510, PerCP-Cy5.5	562970, 562971, 560650
CD25	2A3, MA251	Hu	PE, BD Horizon™ BV421	557138, 564033, 562442
CD45RA	HI100	Hu	PE-Cy™7	560675
CD3	17A2	Ms	APC-Cy™7, BV421	560590, 564008
CD4	RM4-5	Ms	PerCP-Cy5.5	553050
CD25	3C7	Ms	BV421	564370
Stat1 (pY701)	4a	Hu, Ms	Alexa Fluor® 647	612597
Stat3 (pY705)	4/P-STAT3	Hu, Ms	Alexa Fluor® 647	557815
Stat4 (pY693)	38/p-Stat4	Hu, Ms	Alexa Fluor® 647	558137
Stat5 (pY694)	47/Stat5(pY694)	Hu, Ms	Alexa Fluor® 647	562076, 612599
Stat6 (pY641)	23/Stat6, 18/P-Stat6	Hu, Ms	Alexa Fluor® 647	560000, 612601
AKT (pS473)	M8961	Hu	Alexa Fluor® 647	560343
LAT (pY171)	158-1169	Hu	Alexa Fluor® 647	558518
ERK 1/2 (pT202/ pY204)	20A	Hu	Alexa Fluor® 647	561992
Ki-67	B56	Hu	Alexa Fluor® 647	561126
FoxP3	259D/C7	Hu	Alexa Fluor® 488, PE	560047, 560046
	236A/E7	Hu	Alexa Fluor® 488, PE	561181, 560852
	MF23	Ms	Alexa Fluor® 488, PE	560407, 561181
T-bet	O4-46	Hu, Ms	PE, BD Horizon™ PECF594	561268, 562467
GATA3	L50-823	Hu	PE	560074

Table 1. Reagents tested with BD Pharmingen Transcription Factor Phospho Buffer Set

Note: Not all TF have been tested in BD Pharmingen™ Transcription Factor Phospho Buffer Set. It is expected that reactivity would be similar as in data sheet for BD Pharmingen™ Transcription Factor Buffer Set (Catalog 562574, 562725).

Ordering Information

Description	Size	Cat.No.
BD Pharmingen Transcription Factor Phospho Buffer Set	25 Tests	**565575**
	100 Tests	**563239**

23-17476-01

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