

BD Pharmingen™ Transcription Factor Buffer Set

Features

Optimized fixation and permeabilization buffer for the detection of intracellular and intranuclear proteins by flow cytometry

Improved sensitivity for many transcription factors including FoxP3, ROR γ t, Bcl-6, and T-bet

Fully integrated novel formulas speed processing and deliver high cell yield

Compatible with many cell types, cell surface staining, and tandem dyes

Supports high-throughput testing via volume reduction, bulk fixing, and storage

Detection of Transcription Factors by Flow Cytometry

Flow cytometry is a powerful technique for the simultaneous detection of several proteins at the single-cell level. Combining intracellular markers such as transcription factors with cell surface markers allows the determination of subsets within a heterogeneous population expressing the proteins of interest.

While staining with antibodies to cell surface markers is well established, staining intracellular antigens can be more challenging. The BD Pharmingen™ Transcription Factor Buffer permeabilizes the cells sufficiently to allow the exposure of intranuclear epitopes, including FoxP3, which typically requires a unique buffer system that cannot be shared with other markers. Conditions are gentle enough to allow the detection of most cell surface proteins.

Improves Detection of FoxP3, ROR γ t, T-bet, and Other Transcription Factors

The Transcription Factor Buffer Set can be used for the detection of both human and mouse FoxP3, the master transcription factor for regulatory T cells, mouse ROR γ t, and Bcl-6. It is also compatible with many cytokines including interferon- γ , IL-4, and IL-17A.

Please see the table on the reverse side for a complete list of specificities and formats that have been tested.

Novel Formulation for Speed and Ease of Use

The Transcription Factor Buffer Set consists of 4X transcription factor (TF) fixation/permeabilization buffer, a TF 0.75X diluent, and the TF 5X permeabilization/wash buffer.

To ensure ease of use, the Transcription Factor Buffer Set requires just two incubation steps: a 40-minute fix/perm incubation followed by perm/washing, and a 40-minute incubation with monoclonal antibodies followed by final perm/washes. No additional incubation time is needed for permeabilization. Surface staining can be combined with intracellular staining for added convenience.

Supports High-Throughput Testing

To facilitate high-throughput testing, volumes can be reduced to 200 μ L per test. Cells can also be bulk fixed and stored prior to staining for 24 to 48 hours without washing.

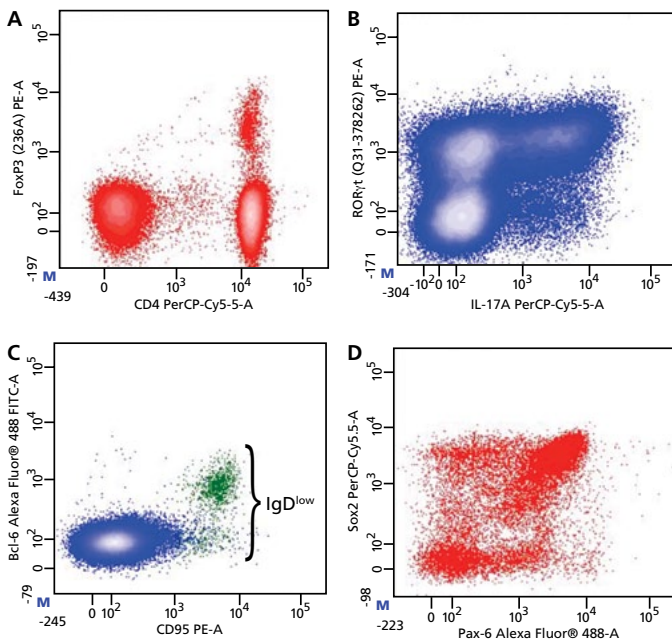


Figure 1. Transcription factor staining by flow cytometry in four different cell preparations using the BD Pharmingen Transcription Factor Buffer Set (Cat. No. 562574).

(A) Expression of FoxP3 in human regulatory T cells. (B) ROR γ t and IL-17A expression in BALB/C mouse polarized Th17 cells. (C) Bcl-6 expression in mouse lymph node and identification of germinal center B cells using the CD4⁻B200⁺IgD^{lo}CD95^{hi} phenotype. (D) Pax6 and Sox2 expression in H9 (WiCell, WI) human embryonic stem cell (ESC)-derived neural cultures.

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Antibodies Tested in Transcription Factor Buffer

Description	Clone	Reactivity	Formats Tested	Cat. No.
Bcl-2	Bcl-2/100	Hu	FITC, PE, BD Horizon™ V450	556357, 556535, 560637
Bcl-6	K22-91	Hu, Ms	Alexa Fluor® 488, Alexa Fluor® 647	561524, 561525
FoxP3	259D/C7	Hu	Alexa Fluor® 488, Alexa Fluor® 647, PE, BD Horizon V450	560047, 560889, 560046, 560459
	236A/E7	Hu	Alexa Fluor® 488, Alexa Fluor® 647, PE, BD Horizon V450, PerCP-Cy™5.5	561181, 561184, 560852, 561493
	MF23	Ms	Alexa Fluor® 488, Alexa Fluor® 647, PE	560403, 560408
GATA3	L50-823	Hu, Ms	PE	560074
H2AX (pS139)	N1-431	Hu, Ms	Alexa Fluor® 488, Alexa Fluor® 647	560445, 560447
IFN-γ	B27	Hu	FITC	554700
	XMG1.2	Ms	APC, FITC	554411, 554413
IL-4	8D4-8	Hu	APC	561233
IL-17A	N49-653	Hu	PE	560487, 560486
	TC11-18H10	Ms	PerCP-Cy5.5	560666
Ki-67	B56	Hu	Alexa Fluor® 488, APC, FITC, PE	561165, 558616, 556003, 556027
Nanog	M55-312	Ms	PE	560277
Nestin	25/NESTIN	Hu, Rat	Alexa Fluor® 647	560393
Oct3/4	40/Oct3	Hu	PerCP-Cy5.5	560794
Pax6	O18-1330	Hu	Alexa Fluor® 488	561664
RORγt	Q31-378	Ms	PE	562607
Sat-β1	14	Hu	Alexa Fluor® 647	562378
Sox1	N23-844	Hu	PE, PerCP-Cy5.5	561592, 561549
Sox2	245610	Hu, Ms	Alexa Fluor® 647, PerCP-Cy5.5	560294, 561506
T-bet	4B10	Hu	PE	561264, 561262
	O4-46	Hu	PE	561268
Themis	Q13-1103	Hu	PE	562588

All formats listed were tested and found to work in the transcription factor buffer. This buffer is likely to work with other formats of the same clones as well as other antibodies. However, they have not been tested.

Ordering Information

Description	Size	Cat. No.
BD Pharmingen Transcription Factor Buffer Set	25 tests	562725
	100 tests	562574

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