Technical Data Sheet

Transcription Factor Buffer Set

Product Information

Material Number:	**562574**
Size:	100 tests
Component:	**51-9008100**
Description:	TF Fix/Perm Buffer (4X)
Size:	25 ml (1 ea)
Component:	**51-9008101**
Description:	TF Diluent Buffer
Size:	75 ml (1 ea)
Component:	**51-9008102**
Description:	TF Perm/Wash Buffer (5X)
Size:	150 ml (1 ea)

Description

The BD Pharmingen™ Transcription Factor Buffer Set is optimized for fixing and permeabilizing cells prior to immunofluorescent staining and flow cytometric analysis of cells that express specific intracytoplasmic and intranuclear proteins. The BD Pharmingen™ Transcription Factor Buffer Set was designed to improve ease-of-use and minimize processing time, to reduce nonspecific staining, to increase the resolution of positively stained cells and to significantly reduce cell loss during fixation, permeabilization and staining procedures. Flow cytometric detection of the many proteins known to be expressed within various intracellular compartments, especially transcription factors, is improved with BD Pharmingen™ Transcription Factor Buffer Set use. This buffer system has been found useful for fixing and permeabilizing a variety of cell types from diverse human and mouse tissues. The buffer system is flexible in supporting multiwell-plate high-throughput and bulk sample analyses and applications that require overnight sample storage. The buffer system has minimal impact on the light-scatter and autofluorescence characteristics of processed cells resulting in characteristics similar to those observed for freshly prepared, highly viable primary cells. In many cases the buffer system was found to be compatible with the immunofluorescent staining of cell-surface antigens both before and after cellular fixation and permeabilization. The buffer system is also compatible with many tandem fluorochromes.

Warnings and Precautions:

- R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
- R36/37/38 Irritating to eyes, respiratory system and skin.
- R40 Limited evidence of a carcinogenic effect.
- R43 May cause sensitization by skin contact.
- S3 Keep in cool place.
- S9 Keep container in a well-ventilated place.
- S23 Do not breathe gas/fumes/vapor/spray.
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
- S60 This material and its container must be disposed of as hazardous waste.

• Caution: The 5x Perm/Wash Buffer contains ≤0.09% sodium azide. Sodium azide yields highly toxic hydrazoic azid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing. The 4x Fix/Perm Buffer contains diethylene glycol and formaldehyde and is harmful.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Recommended Assay Procedure:

Buffer Storage

• Keep stock buffers and 1x working solutions at 2-8°C. After opening the BD Pharmingen™ Transcription Factor Buffer Set, use it within six months. If stored without opening, then use before the expiration date indicated on the bottle labels.

Prior to intracellular staining

• Prepare single-cell suspensions from lymphoid tissues of interest (eg, human peripheral blood, mouse thymus or lymph node). Label 5-ml round-bottom 12 × 75-mm polystyrene tubes and identify appropriate antibodies for your experiment.

• Slowly invert five times the stock BD Pharmingen™ TF Fix/Perm Buffer (4X), TF Diluent Buffer and TF Perm/Wash Buffer (5X) bottles before making working solutions.

• Dilute the 4x Fix/Perm Buffer using the TF Diluent Buffer to the necessary volume of 1x Fix/Perm working solution (a typical dilution for 20 tests is 5 ml of 4x Fix/Perm and 15 ml of TF Diluent Buffer). Use the 1x Fix/Perm Buffer working solution for the Intracellular Staining Protocol listed below within an hour of preparation.

• Dilute the 5x Perm/Wash Buffer to a 1x Perm/Wash Buffer working solution. (A typical dilution for 20 tests would be 30 ml of 5x Perm/Wash Buffer added to 120 ml of deionized water to yield 150 ml of 1x Perm/Wash Buffer). Use the 1x Perm/Wash Buffer working solution for the Intracellular Staining Protocol listed below. Store the 1x Perm/Wash Buffer at 2-8°C for up to one week.

• Buffers for intracellular staining should be kept on ice or at 2-8°C throughout the Intracellular Staining Protocol.

• Surface Staining: Prepare cell suspension containing 10 million cells per ml in flow cytometry stain buffer, such as BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or Stain Buffer (BSA) (Cat. No. 554657). Incubate 100 µl of cells per tube with fluorescent antibodies (eg, antibodies specific for CD4, CD8, CD25, CD19) for 30 minutes at 2-8°C. Wash cells one time with 2 ml of stain buffer and move forward with the intracellular staining protocol listed below.

Intracellular Staining Protocol

1. Fix/Perm: After the cell surface staining procedure is completed, aspirate residual stain buffer. Resuspend cell pellets by brief vortexing and add 1 ml of freshly prepared 1x Fix/Perm Buffer working solution to each tube. Vortex samples for approximately three seconds after adding the Fix/Perm Buffer. Incubate samples at 2-8°C for 40-50 minutes protected from light.

2. Perm/Wash: Add 1 ml of 1x Perm/Wash Buffer directly to the fixed and permeabilized cells suspended in the 1x Fix/Perm Buffer. Pellet the cells by centrifugation. (Note: All centrifugation steps post Fix/Perm are at 350*g* and at 2-8 °C for 6 minutes). Decant or aspirate the supernatants.

3. Perm/Wash: Add 2 ml of 1x Perm/Wash Buffer to the pelleted cells followed by centrifugation. Decant or aspirate wash buffer.

4. Intracellular Staining: Add 80-100 µl of 1x Perm/Wash Buffer to cell samples and the fluorescent antibodies specific for intracellular proteins (eg, FoxP3, T-bet and/or IL-17A) and for nonspecific control staining (eg, matching fluorescent Ig isotype controls) to each tube. Vortex tube or rack for 10 seconds and incubate at 2-8°C for 40-50 minutes protected from light.

5. Perm/Wash: Briefly vortex samples prior to washing. Wash cells with 2 ml of 1x Perm/Wash Buffer. Centrifuge cells. Decant or aspirate the wash buffer.

6. Perm/Wash: Wash cells with 2 ml 1x Perm/Wash. Centrifuge cells. Decant or aspirate wash buffer.

7. Sample preparation for flow cytometry: Resuspend cell pellet in 350 µl of flow cytometry stain buffer. Analyze the cells and acquire data using a flow cytometer.

Notes:

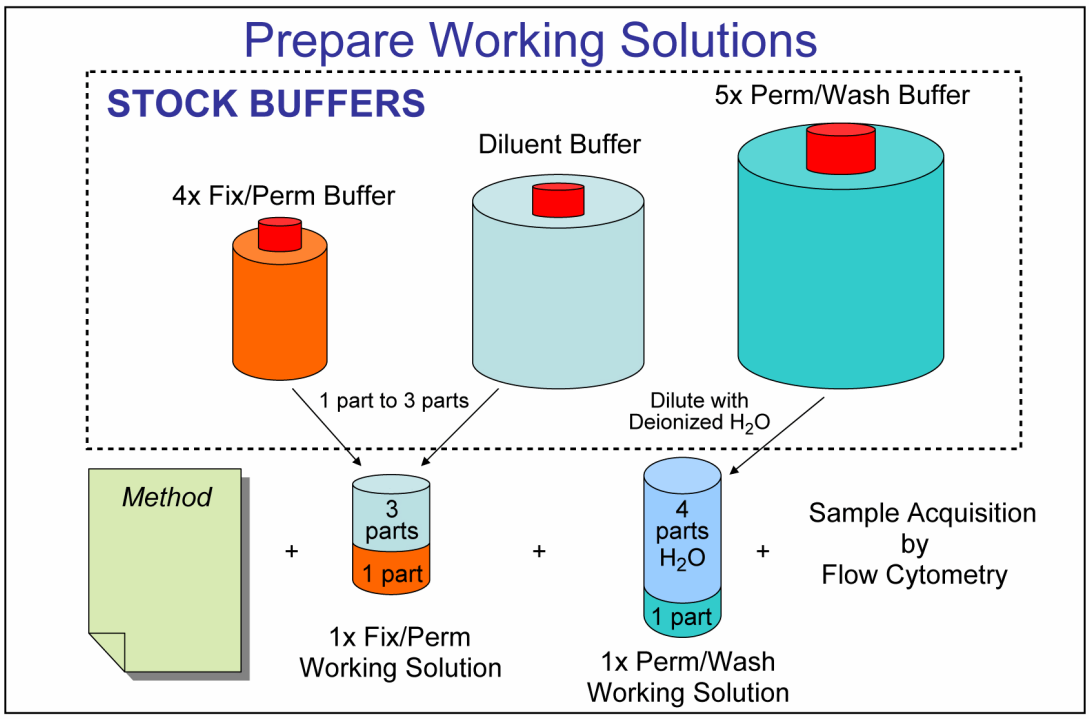
• Due to the fixation and permeabilization procedure, forward and side light-scatter signals will be slightly different than those of live cells.
• The buffer system is optimized for use with fluorescence settings established by using the BD™ Cytometer Setup & Tracking Beads Kit (Cat. No. 642412). However, for your application, minor adjustments in gate and/or detector voltage may need to be made prior to compensation and acquisition.
• Target the acquisition for a statistically significant number of events.
• A titration of the fluorescent antibody's optimal staining amount and optimization of the staining time may be required in your application.

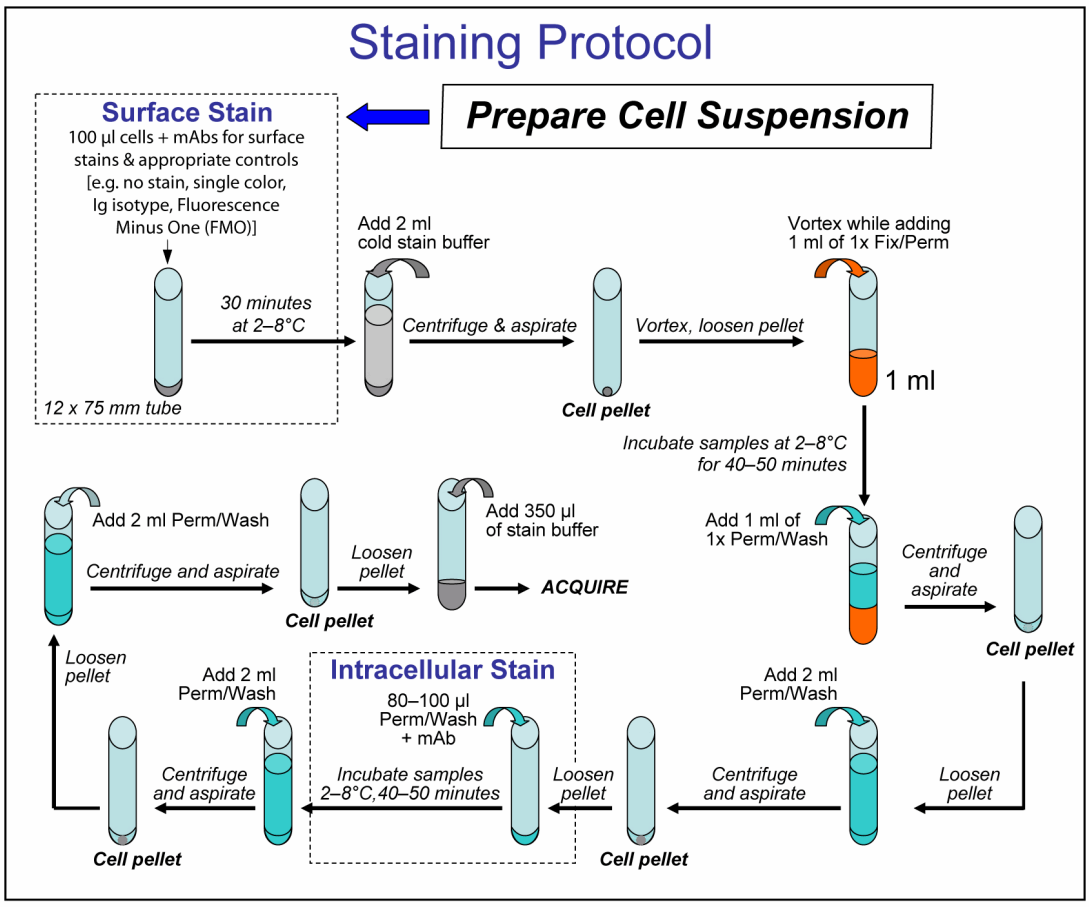
BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**





Overview of Buffer Dilution and Staining Protocol.

BD Biosciences

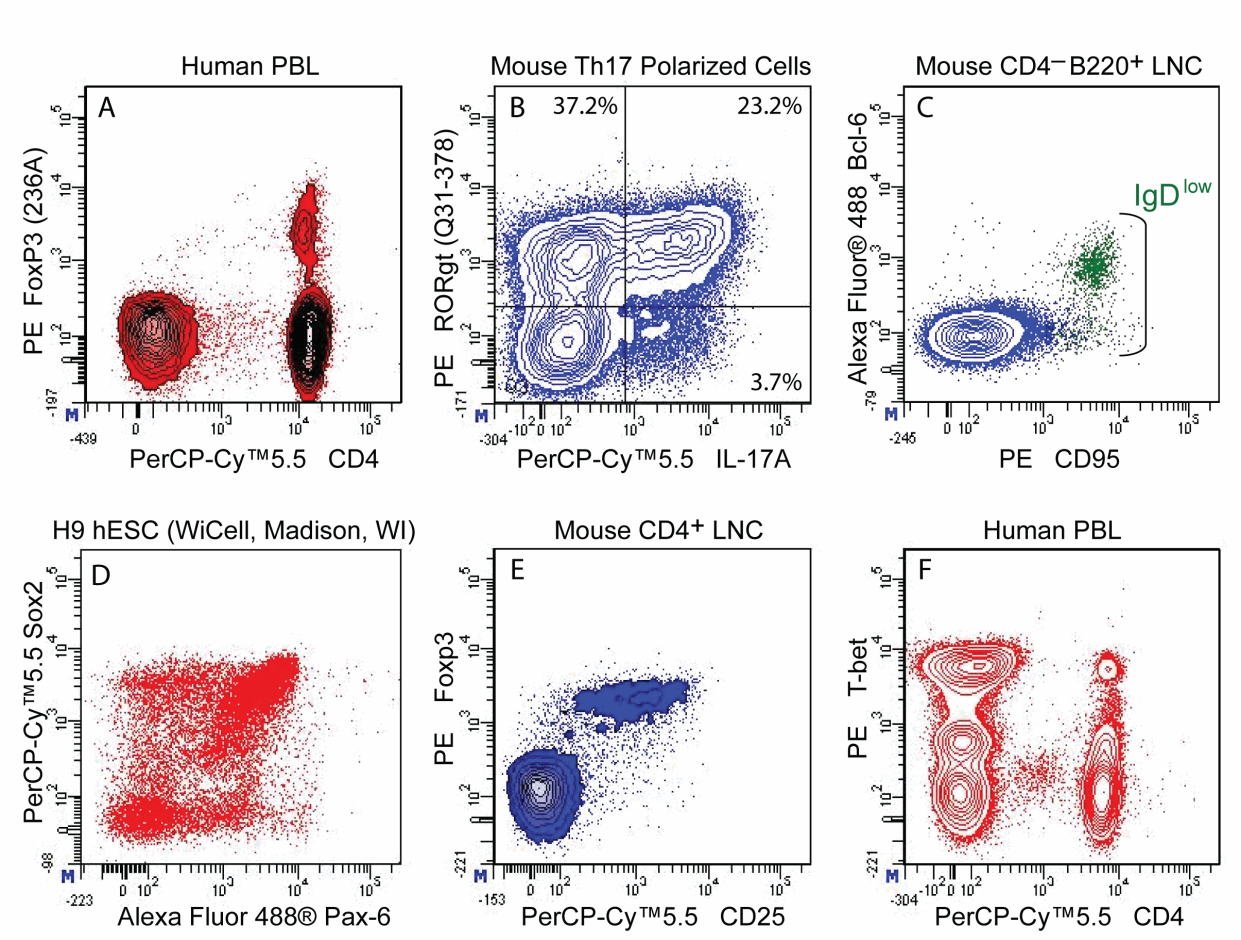
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)
562725	Transcription Factor Buffer Set	25 tests	(none)



Multicolor flow cytometric analysis of transcription factors expressed in different cell types using the BD Pharmingen™ Transcription Factor Buffer Set. *Bivariate flow cytometric plots showing (A) CD4 versus FoxP3 expression in human peripheral blood lymphocytes (PBL); (B) IL-17A versus RORgt expression in BALB/c mouse Th17-polarized cells; (C) CD95 versus Bcl-6 expression in C57BL/6 mouse lymph node cells (LNC) and identification of germinal center B-cells using CD4-B220+IgDloCD95hi phenotype as green colorized dots; (D) Pax-6 versus Sox-2 in H9 (WiCell, Madison, Wi) human embryonic stem cell (ESC) derived neural cultures; (E) mouse CD25 versus Foxp3 expression in CD4 T cells derived from C57BL/6 mouse LNC; (F) CD4 versus T-bet expression in human PBL. Plots were derived from gated events with the forward and side light-scattering characteristics of intact lymphocytes or indicated cell types using BD FACSDiva™ Software v. 6.1.3 and a BD LSRFortessa™ Flow Cytometer System.*

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. Cy is a trademark of Amersham Biosciences Limited.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Antibodies Tested as Compatible for Staining and Flow Cytometric Analysis using the BD Pharmingen™ Transcription Factor Buffer Set

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, 561182, 561493
	MF23	Ms	Alexa Fluor® 488, Alexa Fluor® 647, PE	560403, 560401, 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, FITC, PE, Alexa Fluor® 647	561165, 556026, 556027, 561126
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
SATB1	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	O4-46	Hu, Ms	PE	561268
	4B10	Hu, Ms	PE	561265
THEMIS	Q13-1103	Hu	PE	562588
XBP-1S	Q3-695	Hu, Ms	PE	562642

All formats of the antibodies listed were tested and found to work in staining cells using the BD Pharmingen™ Transcription Factor Buffer Set. This buffer is likely to work with other formats of the same clones as well as other antibodies. However, they have not been tested.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**