Technical Data Sheet

PE-CF594 Mouse Anti-Stat3 (pY705)

Product Information

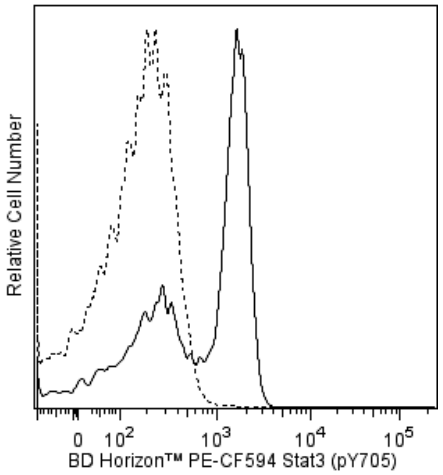
Material Number:	**562673**
Alternate Name:	Acute-phase response factor, APRF
Size:	50 tests
Vol. per Test:	5 µl
Clone:	4/P-STAT3
Immunogen:	Phosphorylated Human Stat3 Peptide
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human; Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Stat (**S**ignal **t**ransducer and **a**ctivators of **t**ranscription) proteins are critical mediators of the biologic activity of cytokines, including interleukins, interferons, erythropoietin, and growth factors. Ligand-receptor interaction leads to activation of constitutively associated JAK family kinases and subsequent recruitment/activation of Stat proteins by tyrosine phosphorylation. Active Stat proteins then move to the nucleus to promote transcription of cytokine-inducible genes. Seven Stat proteins have been cloned, each of which is differentially expressed and/or activated in a cytokine-specific and cell type-specific manner. Stat3 is a 92-kDa protein that is activated as a DNA- binding protein through cytokines, such as IL-6, and growth factors, such as EGF. Stat3 activation occurs via tyrosine phosphorylation at Y705. Tyrosine phosphorylation in response to cytokine stimulation is generally mediated by JAK1. Upon activation, Stat3 dimerizes, translocates to the nucleus and binds DNA response elements, thereby regulating gene expression. It has been reported that Stat3 binds to DNA as a homodimer, but it is also capable of binding as a heterodimer with Stat1. In addition to tyrosine phosphorylation, Stat3 is also phosphorylated at S727 via the MAPK pathway. Stat3 is widely expressed and can bind to the sis-inducible element (SIE) site from the c-fos promoter. This site is similar to the GAS element that is present in IFN-γ induced genes. Thus, phosphorylation of Y705 in Stat3 occurs in response to growth factors and cytokines, and is essential for normal transcription activity.

The 4/P-STAT3 monoclonal antibody recognizes the phosphorylated Y705 of Stat3.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



***Analysis of Stat3 (pY705) expressed in human peripheral blood lymphocytes**. Whole blood was either left unstimulated (dashed line histogram) or stimulated (solid line histogram) with 100 ng/ml BD Pharmingen™ Recombinant Human IL-6 (Cat. No. 550071) for 15 minutes at 37°C. Cells were lysed and fixed in a single step using BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10 min at 37 °C. Cells were then permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for 30 minutes and then stained with BD Horizon™ PE-CF594 anti-Stat3 (pY705) mAb (Cat. No. 562673). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC	human IL-6	Cytofix	Perm III	Upregulated expression
		PBMC	human IL-6	Cytofix	Perm I or II	Unsatisfactory
		Whole Blood	human IL-6	Lyse/Fix	Perm III	Upregulated expression
		Whole Blood	human IL-6	Lyse/Fix	Perm I or II	Unsatisfactory
		U937	human IL-6	Cytofix	Perm III	Upregulated expression
		U937	human IL-6	Cytofix	Perm I or II	Unsatisfactory
WB	Human	A-431	EGF			92-kDa band induced
		U937	human IL-6			92-kDa band induced

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
550071	Recombinant Human IL-6	10 µg	(none)
612356	Purified Mouse Anti-Stat3 (pY705)	50 µg	4/P-STAT3
612357	Purified Mouse Anti-Stat3 (pY705)	150 µg	4/P-STAT3

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. 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.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. CF™ is a trademark of Biotium, Inc.
9. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
10. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
11. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
12. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
13. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Bromberg J, Darnell JE. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene.* 2000; 19(21):2468-2473. (Biology)
Imada K, Leonard WJ. The Jak-STAT pathway. *Mol Immunol.* 2000; 37:1-11. (Biology)
Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signaling by cytokine receptors. *Curr Opin Immunol.* 1998; 10(3):271-278. (Biology)
Renner ED, Rylaarsdam S, Anover-Sombke S, et al. Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced T(H)17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome. *J Allergy Clin Immunol.* 2008; 122(1):181-187. (Clone-specific: Flow cytometry)
Tanaka S, Saito Y, Kunisawa J, Kurashima Y, Wake T, Suzuki N, Shultz LD, Kiyono H, Ishikawa F. Development of mature and functional human myeloid subsets in hematopoietic stem cell-engrafted NOD/SCID/IL2rgammaKO mice. *J Immunol.* 2012; 188(12):6145-6155. (Clone-specific: Flow cytometry)

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