Technical Data Sheet

PE-CF594 Mouse Anti-Stat5 (pY694)

Product Information

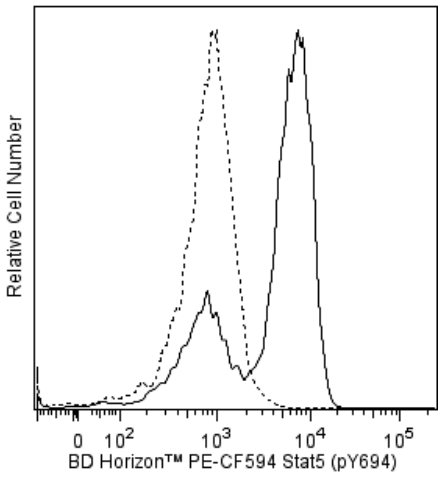
Material Number:	**562501**
Alternate Name:	Signal transducer and activator of transcription 5; MGF; MPF
Size:	50 tests
Vol. per Test:	5 µl
Clone:	47/Stat5(pY694)
Immunogen:	Phosphorylated Sheep Stat5 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution 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. Stat5 has been characterized and shown to be encoded by two separate genes, Stat5a and Stat5b that share over 90% identity at the amino acid level. Stat5a has been shown to be involved in lactogenesis and mammary development, while Stat5b has been shown to be involved in growth hormone signaling and to play a role in liver gene expression. Both Stat5a and Stat5b share similarities, both are involved in IL-2 induced peripheral T cell proliferation. The peptide hormone, prolactin, binds to the prolactin receptor (PRLR) to initiate the lactogenic response. There are at least three forms of PRLR; however, only the long form is able to activate the 92-kDa Stat5 protein by inducing phosphorylation at Y694. Once phosphorylated, Stat5 becomes an essential transcription factor which binds to the β-casein gene promoter. The presence of an SH2 domain within Stat5 suggests that it may directly interact with protein tyrosine kinases (PTKs) such as JAK2.

The 47 monoclonal antibody recognizes the phosphorylated Y694 of Stat5a. The homologous phosphorylation site in Stat5b is Y699.

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).



Flow cytometric analysis of Stat5 (pY694) expression in IL-2-treated human peripheral blood lymphocytes. *Human whole blood was either stimulated with 100 ng/ml (final concentration) of BD Pharmingen™ Recombinant Human IL-2 (Cat. No. 554903) for 15 minutes at 37ºC (solid line histogram) or was left unstimulated (dashed line histogram). The erythrocytes were lysed and the leukocytes were fixed with 1× BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 15 minutes at 37ºC. The leukocytes were washed, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, washed and then stained with BD Phosflow™ PE-CF594 anti-Stat5 (pY694) (Cat. No.562501). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (using BD Phosflow™ Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer or BD Phosflow™ Fix Buffer I).

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

Method	Species	Cells	Treatment	Fixation	Perm buffer	Result
Flow	Human	PBMC	IL-2	Fixation Buffer	III	Positive Staining
Flow	Human	PBMC	IL-2	Fixation Buffer	I or II	Unsatisfactory
Flow	Human	Whole Blood	IL-2	Lyse/Fix	III	Positive Staining
Flow	Human	Whole Blood	IL-2	Lyse/Fix	I or II	Unsatisfactory
WB	Human	A431 Cell Lysate	EGF	Not Applicable	Not Applicable	92 kDa

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)
554603	Recombinant Human IL-2	10 µg	(none)

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
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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.

References

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Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signaling by cytokine receptors. *Curr Opin Immunol.* 1998; 10(3):271-278. (Biology)

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