Technical Data Sheet

PE-Cy™7 Mouse anti-Stat5 (pY694)

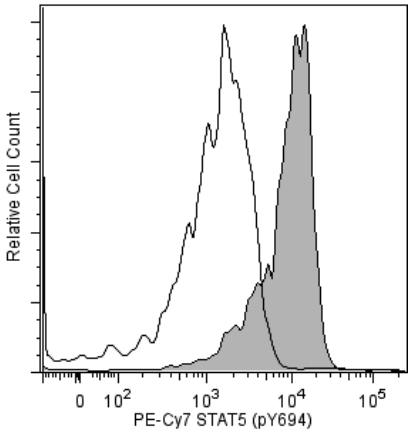
Product Information

Material Number:	**560117**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	47/Stat5(pY694)
Immunogen:	Phosphorylated Sheep Stat5 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed: Human Predicted: Sheep, Rat, 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. 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.



Analysis of Stat5 (pY694) in in human peripheral blood lymphocytes. *Whole blood was either left untreated (unshaded) or treated (shaded) with 100 ng/ml (final concentration) of BD Pharmingen™ Recombinant Human IL-2 (Cat. No. 554903) for 15 minutes at 37°C. The samples were lysed and fixed with 1X BD™ Phosflow Lyse/Fix buffer (Cat. No. 558049) for 10 minutes at 37°C, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes and were then stained with PE-CY™7 anti-Stat5 (pY694). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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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 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
554603	Recombinant Human IL-2	10 µg	(none)
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(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. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
3. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
4. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
5. 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.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Imada K, Leonard WJ. The Jak-STAT pathway. *Mol Immunol.* 2000; 37:1-11. (Biology)
Riou C, Yassine-Diab B, Van grevenynghe J, et al. Convergence of TCR and cytokine signaling leads to FOXO3a phosphorylation and drives the survival of CD4+ central memory T cells.. *J Exp Med.* 2007; 204(1):79-91. (Clone-specific: Flow cytometry)