

Technical Data Sheet

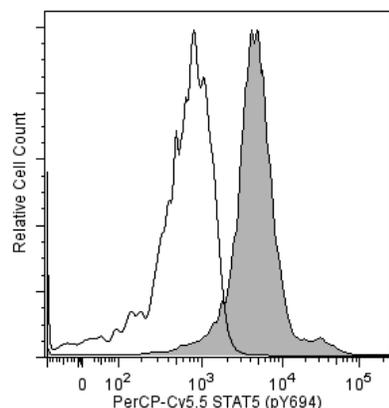
PerCP-Cy™ 5.5 Mouse anti-Stat5 (pY694)**Product Information**

Material Number:	560118
Alternate Name:	Signal transducer and activator of transcription 5; MGF; MPF
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	47/Stat5(pY694)
Immunogen:	Phosphorylated Human Phosphorylated Stat5 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Predicted Reactivity: Mouse, Rat, Sheep
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Stat (Signal transducer and activators of transcription) 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 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 PerCP-Cy™ 5.5 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

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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes**Application**

Intracellular staining (flow cytometry)

Routinely Tested

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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 Cytotfix™ 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. PerCP-Cy5.5-labelled antibodies can be used with FITC- and R-PE-labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
5. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
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 Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. An isotype control should be used at the same concentration as the antibody of interest.

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)

Gouilleux F, Wakao H, Mundt M, Groner B. Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *EMBO J*. 1994; 13(18):4361-4369. (Biology)

Imada K, Leonard WJ. The Jak-STAT pathway. *Mol Immunol*. 2000; 37:1-11. (Biology)

Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S. STAT5 is a potent negative regulator of TFH cell differentiation. *J Exp Med*. 2012; 209(2):243-250. (Clone-specific: Flow cytometry)

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

Prlc M, Bevan MJ. Exploring regulatory mechanisms of CD8+ T cell contraction. *Proc Natl Acad Sci U S A*. 2008; 105(43):16689-16694. (Clone-specific: Flow cytometry)

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)

Suni MA, Maino VC. Flow cytometric analysis of cell signaling proteins. *Methods Mol Biol*. 2011; 717:155-169. (Clone-specific: Flow cytometry)

Van De Wiele CJ, Marino JH, Murray BW, Vo SS, Whetsell ME, Teague TK. Thymocytes between the -Selection and Positive Selection Checkpoints Are Nonresponsive to IL-7 as Assessed by STAT-5 Phosphorylation. *J Immunol*. 2004; 172(7):4235-4244. (Biology)

Wakao H, Gouilleux F, Groner B. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *EMBO J*. 1994; 13(9):2182-2191. (Biology)

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