Technical Data Sheet

PE Mouse Anti-Stat5 (pY694)

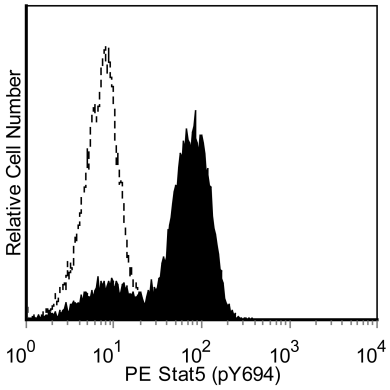
Product Information

Material Number:	**562077**
Alternate Name:	Mammary gland factor, MGF, MPF
Size:	250 tests
Vol. per Test:	5 µl
Clone:	47/Stat5(pY694)
Immunogen:	Phosphorylated Sheep Stat5 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Predicted by immunogen sequence identity: Mouse, Rat, Sheep
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, 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 human lysed whole blood. *Whole blood was either left untreated (unshaded) or treated with 100 ng/mL recombinant human IL-2 (Cat. No. 554603) for 15 minutes at 37°C (shaded). 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 mouse anti-Stat5 (pY694). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCalibur™ 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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
Flow	Human	TF-1 cells	GM-CSF	Fixation Buffer	III	Positive Staining
Flow	Human	TF-1 cells	GM-CSF	Fixation Buffer	I or II	Unsatisfactory
WB	Human	A431 Cell Lysate	EGF	Not Applicable	Not Applicable	92 kDa

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557870	Fix Buffer I	250 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554603	Recombinant Human IL-2	10 µg	(none)
611964	Purified Mouse Anti-Human Stat5 (pY694)	50 µg	47/Stat5(pY694)

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. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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)
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)