

Technical Data Sheet

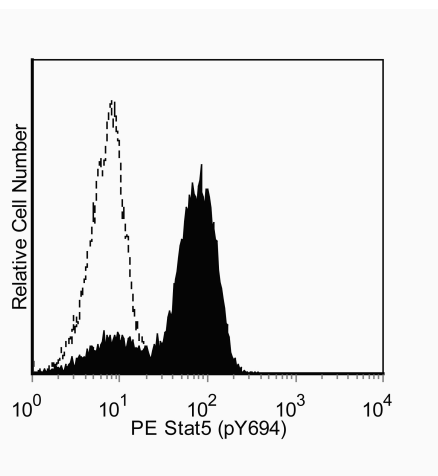
PE Mouse Anti-Stat5 (pY694)**Product Information**

Material Number:	612567
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, protein stabilizer, and ≤0.09% sodium azide.

Description

Stat (Signal transducer and activators of transcription) proteins mediate the biological activity of cytokines, including interleukins, interferons, erythropoietin, and growth factors. Ligand-receptor interaction activates 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, which 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 liver gene expression. Both Stat5a and Stat5b 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 activates 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.



Flow cytometric analysis of Stat5 (pY694) in human lysed whole blood. Whole blood was either left untreated (dashed line histogram) or treated with 100 ng/mL Recombinant Human IL-2 for 15 minutes at 37°C (shaded histogram; Cat. No. 554603). The samples were lysed and fixed with 1X Lyse/Fix Buffer 5X (Cat. No. 558049) for 10 minutes at 37°C, permeabilized (Perm Buffer III; Cat. No. 558050) on ice for 30 minutes and were then stained with PE Mouse Anti-Stat5 (pY694), (Cat. No. 612567). The fluorescence histograms showing STAT5 (PY694) or Ig isotype control staining were derived from gated events with the forward and side light-scattering characteristics of viable lymphocytes. Flow cytometry was performed on a BD FACSCalibur™.

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.

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612567 Rev. 9



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 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
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)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 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. 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 Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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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)