Technical Data Sheet

Alexa Fluor® 488 Anti-Stat5 (pY694)

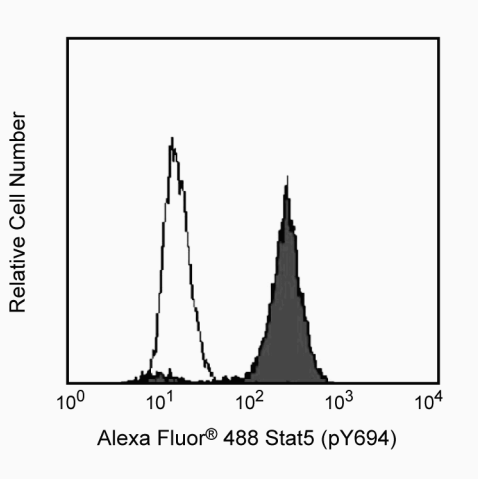
Product Information

Material Number:	**612598**
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 (**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.



Flow cytometric analysis of phospho-Stat5 (pY694). *TF-1 cells (GM-CSF dependent cell line) were starved overnight in IMDM with 0.1% FBS without GM-CSF. The following day, cells were either left unstimulated (unshaded) or stimulated with GM-CSF recombinant protein (Cat. No. 550068) at 25 ng/ml for 15 minutes at 37°C (shaded). Cells were fixed using BD Cytofix™ Fixation Buffer (10 minutes at 37°C) and then permeabilized in BD Phosflow™ Perm Buffer III (30 minutes on ice or overnight at -20°C). Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656), and stained with Alexa Fluor® 488 Anti-Stat5 (pY694) antibody for 1 hour at RT (Cat. No. 612598). The cells were analyzed on a BD FACSCalibur™ flow cytometer.*

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 to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

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 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
550068	Recombinant Human GM-CSF	10 µg	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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