

Technical Data Sheet

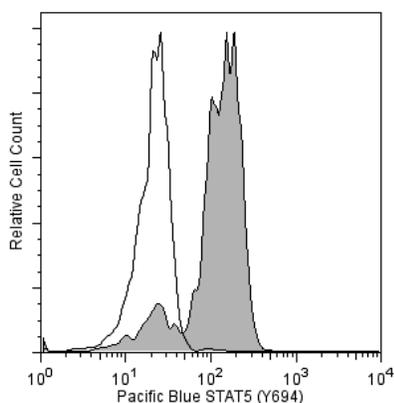
Pacific Blue™ Mouse anti-Stat5 (pY694)**Product Information**

Material Number:	560311
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 Sheep Stat5 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC testing: Human Predicted by immunogen sequence identity: Chimpanzee, 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 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. Human peripheral blood mononuclear cells (PBMC) were either left untreated (unshaded) or treated (shaded) with 100 ng/ml (final concentration) of BD Pharmingen™ Recombinant Human IL-2 (Cat. No. 554603) for 15 minutes at 37°C. The PBMC were fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655) 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 Pacific Blue™ Mouse 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 is conjugated to Pacific Blue™ under optimum conditions, and unreacted Pacific Blue™ was removed.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit bdbiosciences.com/contact

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD

The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	Species	Cells	Treatment	Fixation	Perm buffer	Result
Flow	Human	PBMC	human IL-2	Cytofix	Perm III	Upregulated expression
		PBMC	human IL-2	Cytofix	Perm I or II	Unsatisfactory
		Whole Blood	human IL-2	Lyse/Fix	Perm III	Upregulated expression
		Whole Blood	human IL-2	Lyse/Fix	Perm I or II	Unsatisfactory
		TF-1	human GM-CSF	Cytofix	Perm III	Upregulated expression
		TF-1	human GM-CSF	Cytofix	Perm I or II	Unsatisfactory
WB	Human	A-431	EGF			92-kDa band induced

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 and cell lines (using BD Cytofix™ Fixation Buffer).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554603	Recombinant Human IL-2	10 µg	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

- 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).
- Pacific Blue™ has a maximum absorption of 416 nm and maximum emission of 451 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 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.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 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.
- Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
- Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

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)
 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)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit bdbiosciences.com/contact

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD

