Technical Data Sheet

Pacific Blue™ Mouse anti-Stat3 (pY705)

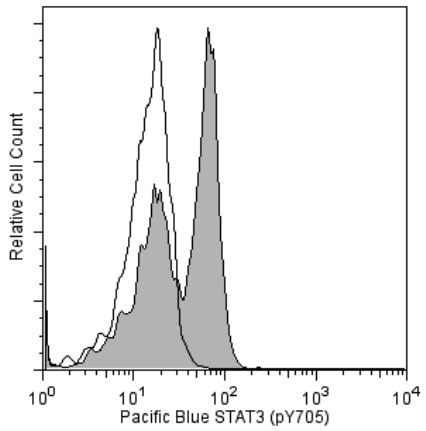
Product Information

Material Number:	**560312**
Alternate Name:	Acute-phase response factor, APRF
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	4/P-STAT3
Immunogen:	Phosphorylated Human Stat3 Peptide
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse Predicted Reactivity: Chimpanzee, Cow
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. Stat3 is a 92-kDa protein that is activated as a DNA- binding protein through cytokines, such as IL-6, and growth factors, such as EGF. Stat3 activation occurs via tyrosine phosphorylation at Y705. Tyrosine phosphorylation in response to cytokine stimulation is generally mediated by JAK1. Upon activation, Stat3 dimerizes, translocates to the nucleus and binds DNA response elements, thereby regulating gene expression. It has been reported that Stat3 binds to DNA as a homodimer, but it is also capable of binding as a heterodimer with Stat1. In addition to tyrosine phosphorylation, Stat3 is also phosphorylated at S727 via the MAPK pathway. Stat3 is widely expressed and can bind to the sis-inducible element (SIE) site from the c-fos promoter. This site is similar to the GAS element that is present in IFN-γ induced genes. Thus, phosphorylation of Y705 in Stat3 occurs in response to growth factors and cytokines, and is essential for normal transcription activity.

The 4/P-STAT3 monoclonal antibody recognizes the phosphorylated Y705 of Stat3.



Analysis of Stat3 (pY705) in human peripheral blood lymphocytes. *Whole blood was either left unstimulated (unshaded) or stimulated (shaded) with 100 ng/ml BD Pharmingen™ Recombinant Human IL-6 (Cat. No. 550071) for 15 minutes at 37°C. The cells were lysed and fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37ºC, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Pacific Blue™ Mouse anti-Stat3 (pY705). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCanto™ II 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 is conjugated to Pacific Blue™ under optimum conditions, and unreacted Pacific Blue™ was removed.

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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-6	Cytofix	Perm III	Upregulated expression
		PBMC	human IL-6	Cytofix	Perm I or II	Unsatisfactory
		Whole Blood	human IL-6	Lyse/Fix	Perm III	Upregulated expression
		Whole Blood	human IL-6	Lyse/Fix	Perm I or II	Unsatisfactory
		U937	human IL-6	Cytofix	Perm III	Upregulated expression
		U937	human IL-6	Cytofix	Perm I or II	Unsatisfactory
WB	Human	A-431	EGF			92-kDa band induced
		U937	human IL-6			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
554655	Fixation Buffer	100 mL	(none)
558049	Lyse/Fix Buffer 5X	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)
550071	Recombinant Human IL-6	10 µg	(none)
612356	Purified Mouse Anti-Stat3 (pY705)	50 µg	4/P-STAT3
612357	Purified Mouse Anti-Stat3 (pY705)	150 µg	4/P-STAT3

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. 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.
4. 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.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. 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.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

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)

Darnell JE Jr. STATs and gene regulation. *Science.* 1997; 277(5332):1630-1635. (Biology)

Fu XY, Zhang JJ. Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-fos gene promoter. *Cell.* 1993; 74(6):1135-1145. (Biology)

Kanai M, Konda Y, Nakajima T, et al. Differentiation-inducing factor-1 (DIF-1) inhibits STAT3 activity involved in gastric cancer cell proliferation via MEK-ERK-dependent pathway. *Oncogene.* 2003; 22(22):548-554. (Biology)

Smith PD, Crompton MR. Expression of v-src in mammary epithelial cells induces transcription via STAT3. *Biochem J.* 1998; 15:331-381. (Biology)

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