Technical Data Sheet

Pacific Blue™ Mouse anti-Stat1 (pY701)

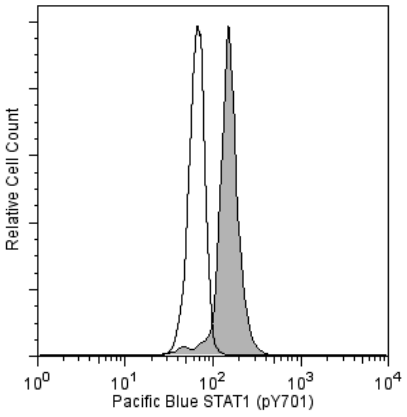
Product Information

Material Number:	**560310**
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	14/P-STAT1
Immunogen:	Phosphorylated Human Stat1 (pY701) Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
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. Stat1 and Stat2 are components of the ISGF3 (**I**nterferon-**S**timulated **G**ene **F**actor **3**) complex, which is the primary transcription activator induced by the binding of the interferon to a specific cell-surface receptor. Stat1 has two alternatively spliced isoforms, 91-kDa Stat1α and 84-kDa Stat1β; Stat1α has 38 additional C-terminal amino acids. In response to the binding of IFNα, IFNγ, EGF, PDGF, or CSF-1 to their respective receptors, the Stat1 subunits become tyrosine-phosphorylated at Y701, and the complex is translocated to the nucleus. This results in the formation of an active complex that includes the DNA-binding p48 subunit. This complex is responsible for modulating the transcription of the interferon-stimulated genes (ISGs).

The 14/P-STAT1 monoclonal antibody recognizes the phosphorylated Y701 in Stat1α and Stat1β.



Analysis of Stat1 (pY701) in human peripheral blood monocytes. *Peripheral blood mononuclear cells (PBMC) were either left unstimulated (unshaded) or stimulated (shaded) with 100 ng/ml (final concentration) BD Pharmingen™ Recombinant Human IFNγ (Cat. No. 554617) for 15 minutes at 37°C. The PBMC were fixed (BD Cytofix™ Fixation buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes and then stained with Pacific Blue™ Mouse anti-Stat1(pY701). For data analysis, monocytes 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 IFNγ	Cytofix	Perm III	Upregulated expression
		PBMC	human IFNγ	Cytofix	Perm I or II	Unsatisfactory
		U937	human IFNγ	Cytofix	Perm III	Upregulated expression
		U937	human IFNγ	Cytofix	Perm I or II	Unsatisfactory
WB	Human	A-431	EGF			84- & 91-kDa bands induced

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554617	Recombinant Human IFN-γ	50 µg	(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. 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.
4. 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.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. 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)

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