Technical Data Sheet

PerCP-Cy™5.5 Mouse anti-Stat1 (pY701)

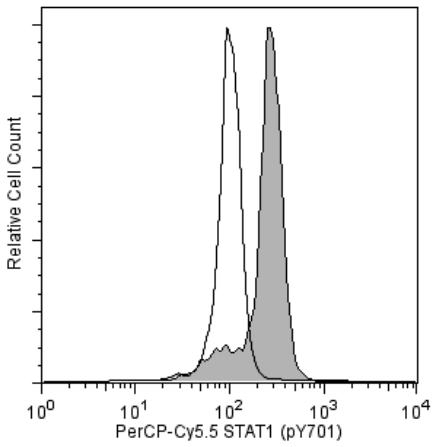
Product Information

Material Number:	**560113**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	4a
Immunogen:	Phosphorylated Human Stat1 Peptide
Isotype:	Mouse IgG2a
Reactivity:	Confirmed by flow cytometry: Human Confirmed by western blot using purified antibody (Cat. No. 612232 or 612233): Mouse Predicted: Rat
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. 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 4a 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 PerCP-CY™5.5 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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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Recommended Assay Procedure:
Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation.

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	IFNγ	Fixation Buffer	III	Positive Staining
Flow	Human	PBMC	IFNγ	Fixation Buffer	I or II	Unsatisfactory
WB	Human	U937 Cell Lysate	IFNγ	Not Applicable	Not Applicable	91/84 kDa
WB	Human	A431 Cell Lysate	EGF	Not Applicable	Not Applicable	91/84 kDa

Suggested Companion Products

Catalog Number	Name	Size	Clone
554617	Recombinant Human IFN-γ	50 µg	(none)
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 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 observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
3. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
4. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
6. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/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)
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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