Technical Data Sheet

PerCP-Cy™5.5 Mouse anti-Stat3 (pY705)

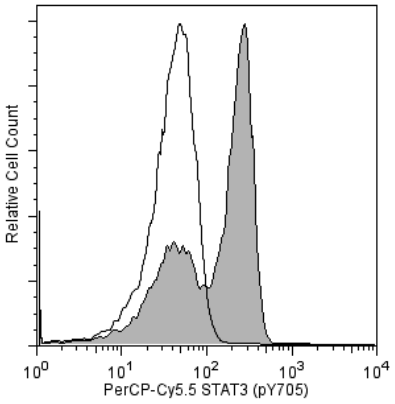
Product Information

Material Number:	**560114**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	4/P-STAT3
Immunogen:	Phosphorylated Human Stat3 Peptide
Isotype:	Mouse IgG2a, κ
Reactivity:	Confirmed by flow cytometry: Human Confirmed by western blot using purified antibody (Cat. No. 612356 or 612357): Mouse, Rat Predicted: Cow
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. 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 samples were fixed (BD Cytofix™ Fixation buffer, Cat. No. 55465) 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-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

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.

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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 (using BD Cytofix™ Fixation Buffer or BD™ Phosflow Fix Buffer I).

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	IL-6	Fixation Buffer	III	Positive Staining
Flow	Human	PBMC	IL-6	Fixation Buffer	I or II	Unsatisfactory
Flow	Human	Whole Blood	IL-6	Lyse/Fix	III	Positive Staining
Flow	Human	Whole Blood	IL-6	Lyse/Fix	I or II	Unsatisfactory
WB	Human	U937 Cell Lysate	IL-6	Not Applicable	Not Applicable	92 kDa
WB	Human	A431 Cell Lysate	EGF	Not Applicable	Not Applicable	92 kDa

Suggested Companion Products

Catalog Number	Name	Size	Clone
550071	Recombinant Human IL-6	10 µg	(none)
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 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

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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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