

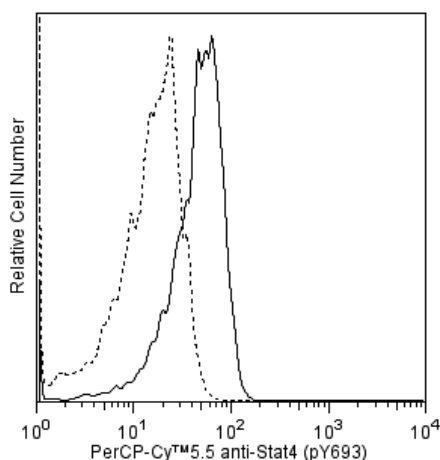
Technical Data Sheet

PerCP-Cy™ 5.5 Mouse Anti-Stat4 (pY693)**Product Information**

Material Number:	561217
Alternate Name:	Signal transducer and activator of transcription 4; SLEB11
Size:	50 tests
Vol. per Test:	5 µl
Clone:	38/p-Stat4
Immunogen:	Phosphorylated Human Stat4 (pY693)
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human Confirmed by western blot in Mouse using Purified Anti-Stat4 (pY693), Cat. No. 612739, 612738
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Stat proteins function both as cytoplasmic signal transducers and as activators of transcription. Seven mammalian Stat proteins have been identified: Stat1-4, Stat5a, 5b, and Stat6. Stat4 has been shown to play an important role in development of T helper cells, specifically the Th1 subset. Stat4 is activated by IL-12 and by type 1 interferons. Knockout mice supported the role that Stat4 plays in IL-12 signaling because lymphocytes from Stat4^{-/-} mice could neither differentiate into Th1 cells nor produce IFN-γ in response to treatment with IL-12. IFN-γ plays an important role in host defense. A key component in the activation of Stat4 is the phosphorylation on tyrosine and serine residues in response to IL-12 stimulation. IL-12 stimulation leads to the phosphorylation of Stat4 on tyrosine 693 and serine 721. Transcriptional activity of Stat4 has been shown to be significantly reduced when residues Y693 and S721 are mutated.

**Flow cytometric analysis of Stat4 (pY693) expression in IFN-α treated human peripheral blood lymphocytes.**

Human whole blood was either stimulated with 40,000 U/ml of IFN-α for 15 minutes at 37°C (solid line histogram) or was left unstimulated (dashed line histogram). The erythrocytes were lysed and the leukocytes were fixed with 1× BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37°C. The leukocytes were washed, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, washed and then stained with PerCP-Cy™ 5.5 anti-Stat4 (pY693) (Cat. No. 561217). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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 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.

Application Notes**Application**

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

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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- μ l experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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 Cy5.5TM. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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References

Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene*. 2002; 285:1-24. (Biology)

Visconti R, Gadina M, Chiariello M, Chen EH, Stancato LF, Gutkind JS, O'Shea J. Importance of the MKK6/p38 pathway for interleukin-12-induced STAT4 serine phosphorylation and transcriptional activity. *Blood*. 2000; 96:1844-1852. (Biology)

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