Technical Data Sheet

PE Mouse Anti-Stat3 (pY705)

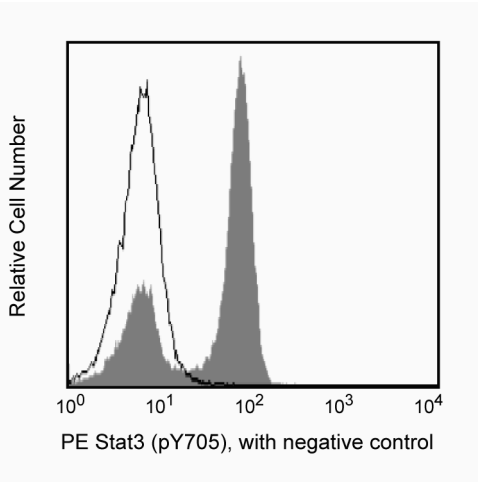
Product Information

Material Number:	**612569**
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
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.



Flow cytometric analysis of Stat3 (pY705). *Human whole blood was collected in the presence of heparin. Whole blood was either left unstimulated (unshaded) or stimulated (shaded) with recombinant human IL-6 (Cat. No. 550071) at 100 ng/mL for 15 min at 37 °C. Cells were lysed and fixed in a single step using BD Phosflow™ Lyse/Fix buffer (Cat. No.558049) for 10 min at 37 °C. Cells were then permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) for 30 min on ice or overnight at -20 °C. Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656) and stained with PE mouse anti-Stat3 (pY705) antibody (Cat. No. 612569) for 30 min at room temperature. Samples were analyzed on a BD FACSCalibur™ instrument.*

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
550071	Recombinant Human IL-6	10 µg	(none)
558595	PE Mouse IgG2a, κ Isotype Control	50 Tests	MOPC-173
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 refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. An isotype control should be used at the same concentration as the antibody of interest.

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)
Imada K, Leonard WJ. The Jak-STAT pathway. *Mol Immunol.* 2000; 37:1-11. (Biology)
Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signaling by cytokine receptors. *Curr Opin Immunol.* 1998; 10(3):271-278. (Biology)
Renner ED, Rylaarsdam S, Anover-Sombke S, et al. Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced T(H)17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome. *J Allergy Clin Immunol.* 2008; 122(1):181-187. (Clone-specific: Flow cytometry)
Tanaka S, Saito Y, Kunisawa J, Kurashima Y, Wake T, Suzuki N, Shultz LD, Kiyono H, Ishikawa F. Development of mature and functional human myeloid subsets in hematopoietic stem cell-engrafted NOD/SCID/IL2rgammaKO mice. *J Immunol.* 2012; 188(12):6145-6155. (Clone-specific: Flow cytometry)

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