Technical Data Sheet

PE Mouse anti-Stat3 (pS727)

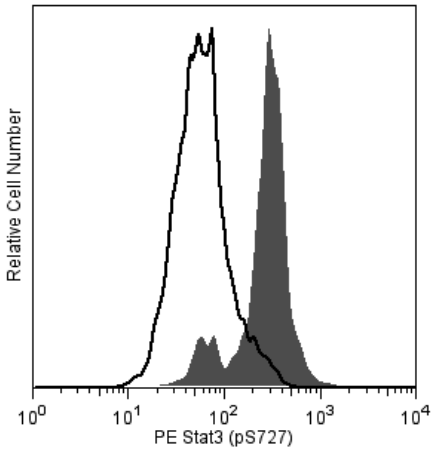
Product Information

Material Number:	**558557**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	49/p-Stat3
Immunogen:	Phosphorylated Human Stat3
Isotype:	Mouse IgG1
Reactivity:	Confirmed by flow cytometry: human Confirmed by western blot using purified antibody (Cat. No. 612542 or 612543): mouse, rat
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. 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 is phophorylated at serine 727 (S727) via the MAPK pathway. The S727 residue is located at a conserved Pro-X-Ser-Pro sequence, which is recognized by the protein kinase ERK. Activation through the S727 residue is thought to lead to initiation of transcription. Upon activation, Stat3 dimerizes, translocates to the nucleus, and binds DNA response elements thereby regulating gene expression. It appears that Stat3 binds to DNA as a homodimer, but it is also capable of binding as a heterodimer with Stat1. In addition to serine phosphorylation, Stat3 is also phosphorylated at tyrosine 705 by JAK1 in response to cytokine stimulation. 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 S727 in Stat3 occurs in response to growth factors and cytokines, and is essential for normal transcription activity.

The 49/p-Stat3 monoclonal antibody recognizes the S727-phosphorylated form of Stat3 (isoform 1). The fluorochrome-conjugated formats have been evaluated using a human model system. However, the unconjugated form of this antibody (Cat. no. 612542 or 612543) is also effective for western blot analysis of human, mouse, and rat tissue.



Analysis of Stat3 (pS727) in monocytes. *Human peripheral blood mononuclear cells (PBMC) were either stimulated with 40 nM PMA (Sigma, P8139) for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD™ PhosFlow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE Mouse anti-Stat3 (pS727). Monocytes were selected by scatter profile. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

Preparation and Storage

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.
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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)

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)

Schuringa JJ, Dekker LV, Vellenga E, Kruijer W. Sequential activation of Rac-1, SEK-1/MKK-4, and protein kinase Cδ is required for interleukin-6-induced STAT3 Ser-727 phosphorylation and transactivation. *J Biol Chem.* 2001; 276:27709-27715. (Biology)