Technical Data Sheet

APC Mouse anti-Stat3

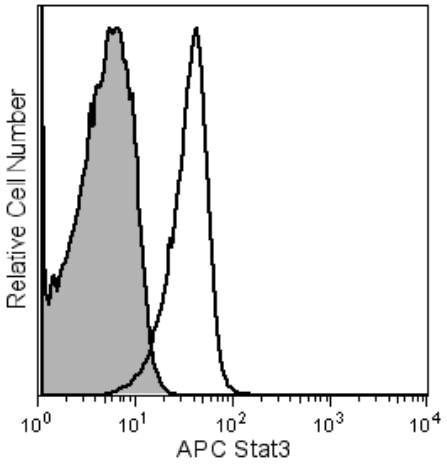
Product Information

Material Number:	**560392**
Alternate Name:	Acute-phase response factor, APRF
Size:	50 tests
Vol. per Test:	20 µl
Clone:	M59-50
Immunogen:	Human Stat3 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, λ
Reactivity:	QC Testing: Human Predicted due to identity of immunogen sequence: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

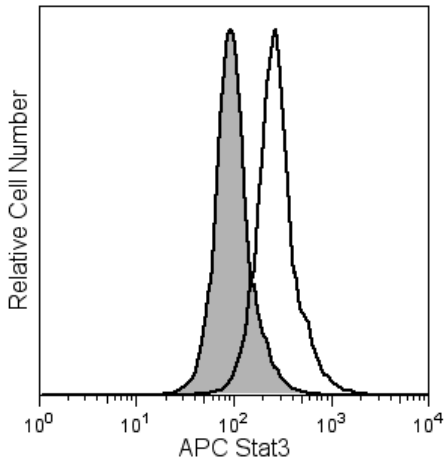
Description

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. 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. 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. 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.

The M59-50 monoclonal antibody recognizes Stat3 (isoform 1) regardless of phosphorylation status. The specificity of this antibody conjugate for flow cytometric analysis was validated by confirming that RNA-mediated interference (RNAi) of the specific protein reduced the staining of the cells (see figure). Furthermore, the capacity of the RNAi to down-regulate the expression of the relevant protein was confirmed by western blot analysis.



Analysis of Stat3 in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37ºC, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with either APC Mouse IgG1, κ Isotype Control (Cat. No. 554681, shaded histogram) or APC Mouse anti-Stat3 (open histogram). For data analysis, lymphocytes were selected by their scatter profile. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*



Analysis of Stat3 in human epithelioid carcinoma. *HeLa S3 cells (ATCC CCL 2.2) were either transfected with Stat3 RNAi (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ 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 APC Mouse anti-Stat3. Flow cytometry was performed on a BD FACSArray™ bioanalyzer system.*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human cell lines and peripheral blood mononuclear cells using BD Cytofix™ Fixation Buffer. Either BD™ Phosflow Perm Buffer II or Perm Buffer III may be used. We have observed that BD™ Phosflow Perm/Wash Buffer I gives unsatisfactory results with this antibody.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554681	APC Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21

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. 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)

Darnell JE Jr. STATs and gene regulation. *Science.* 1997; 277(5332):1630-1635. (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)

Kirito K, Osawa M, Morita H. A functional role of Stat3 in in vivo megakaryopoiesis. *Blood.* 2002; 99(9):3220-3227. (Biology)

Smith PD, Crompton MR. Expression of v-src in mammary epithelial cells induces transcription via STAT3. *Biochem J.* 1998; 15:331-381. (Biology)