

Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Stat1 (pS727)

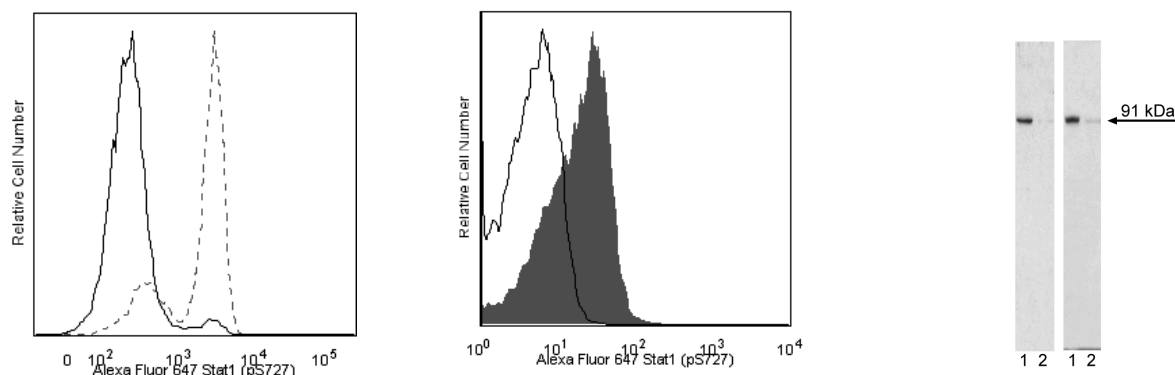
Product Information

Material Number:	560190
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K51-856
Immunogen:	Phosphorylated Human Stat1 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed: Human; Predicted: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Stat (Signal transducer and activators of transcription) 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. Stat1 and Stat2 are components of the ISGF3 (Interferon-Stimulated Gene Factor 3) complex, which is the primary transcription activator induced by the binding of the interferon to a specific cell-surface receptor. Stat1 has two alternatively spliced isoforms, 91-kDa Stat1α and 84-kDa Stat1β; Stat1α has 38 additional C-terminal amino acids. In response to the binding of IFNα, IFNγ, EGF, PDGF, or CSF-1 to their respective receptors, the Stat1 subunits become tyrosine-phosphorylated at Y701, and the complex is translocated to the nucleus. This results in the formation of an active complex that includes the DNA-binding p48 subunit. This complex is responsible for modulating the transcription of the interferon-stimulated genes (ISGs). Furthermore, phosphorylation of serine 727 (S727) of Stat1 may occur in response to pathogens, cytokines, UV irradiation, and engagement of T and B cell antigen receptors. This second phosphorylation event is necessary for fully effective activation of transcription and may differentially regulate gene activation by interacting with other regulatory factors, such as p53 in the apoptotic pathway.

The K51-856 monoclonal antibody recognizes the phosphorylated S727 in the C-terminal transactivating domain of human Stat1α; this site is not present in Stat1β.



Analysis of Stat1 (pS727) in human epithelioid carcinoma and peripheral blood lymphocytes. HeLa S3 cells (left panel, ATCC CCL 2.2) were either treated with 0.5 µg/ml Nocodazole (Sigma-Aldrich, Cat. No. M1404) at 37°C for 18 hours (left panel, dashed line) or untreated (solid line). Human peripheral blood mononuclear cells (middle panel) were either stimulated with 50 nM PMA (Sigma, P8139) for 15 minutes at 37°C (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-Stat1 (pS727). Flow cytometry was performed on a BD™ LSR II (left) or FACSCalibur (middle) flow cytometer, and lymphocytes were identified by their scatter profile. The right panel shows confirmation of the specificity by western blot using unconjugated K51-856 antibody at 2.0 µg/ml on lysates from PMA-treated (lane 1, left blot) and control (lane 2) PBMCs and Nocodazole-treated (lane 1, right blot) and control (lane 2) HeLa S3 cells. Stat1 (pS727) is identified as a band of 91 kDa in the treated cells.

Preparation and Storage

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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

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/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2011 BD



The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	Species	Cells	Treatment	Fixation	Perm buffer	Result
Flow	Human	A-431	UV irradiation	Cytofix	Perm III	Up-regulation
	Human	HeLa S3	UV irradiation or Nocodazole	Cytofix	Perm III	Up-regulation
	Human	PBMC	PMA	Cytofix	Perm I, II, or III	Up-regulation
	Human	U-937	Anisomycin, IFN- γ , or PMA	Cytofix	Perm III	Unsatisfactory
	Human	Whole blood	IFN- α , IFN- γ , or PMA	Lyse/Fix	Perm III	Unsatisfactory
WB	Human	A-431	UV irradiation			91-kDa band induced
	Human	HeLa S3	Nocodazole			91-kDa band induced
	Human	Jurkat	IFN- α			Unsatisfactory
	Human	PBMC	PMA			91-kDa band induced
	Human	U-937	Anisomycin			Unsatisfactory
	Human	U-937	UV irradiation			91-kDa band induced

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
---	------------------

Recommended Assay Procedure:

Either BD Cytofix™ fixation buffer or BD™ Phosflow Fix Buffer I may be used for cell fixation. Any of the three BD™ Phosflow permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
557870	Fix Buffer I	250 ml	(none)
554655	Fixation Buffer	100 ml	(none)

Product Notices

- Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
- 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).
- Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
- For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
- 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.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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)

Gamero AM, Larner AC. Signaling via the T cell antigen receptor induces phosphorylation of Stat1 on serine 727. *J Biol Chem*. 2000; 275(22):16574-16578. (Biology)

Heim MH. The Jak-STAT pathway: specific signal transduction from the cell membrane to the nucleus. *Eur J Clin Invest*. 1996; 26(1):1-12. (Biology)

Kovarik P, Mangold M, Ramsauer K, et al. Specificity of signaling by STAT1 depends on SH2 and C-terminal domains that regulate Ser727 phosphorylation, differentially affecting specific target gene expression. *EMBO J*. 2001; 20(1 & 2):91-100. (Biology)

Nguyen H, Ramana CV, Bayes J, Stark GR. Roles of phosphatidylinositol 3-kinase in interferon- γ -dependent phosphorylation of STAT1 on serine 727 and activation of gene expression. *J Biol Chem*. 2001; 276(36):33361-33368. (Biology)

Townsend PA, Scarabelli TM, Davidson SM, Knight RA, Latchman DS, Stephanou A. STAT-1 interacts with p53 to enhance DNA damage-induced apoptosis. *J Biol Chem*. 2004; 279(7):5811-5820. (Biology)

Varinou L, Ramsauer K, Karaghiosoff M, et al. Phosphorylation of the Stat1 transactivation domain is required for full-fledged IFN- γ -dependent innate immunity. *Immunity*. 2003; 19:793-802. (Biology)