Technical Data Sheet

Alexa Fluor® 647 Mouse Anti-Stat1 (pY701)

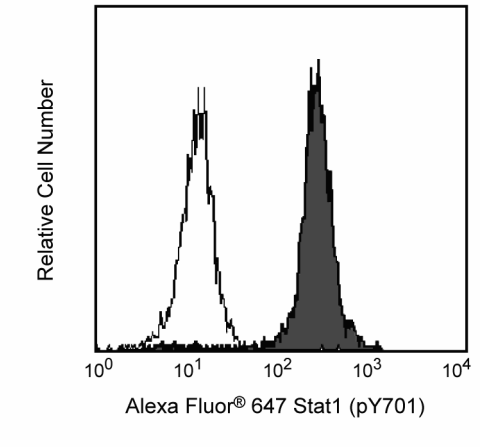
Product Information

Material Number:	**612597**
Alternate Name:	Signal Transducer and Activator of Transcription-1; STAT91; ISGF-3; CANDF7
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	4a
Immunogen:	Phosphorylated Human Stat1 Peptide
Isotype:	Mouse IgG2a
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. Stat1 and Stat2 are components of the ISGF3 (**I**nterferon-**S**timulated **G**ene **F**actor **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).

The 4a monoclonal antibody recognizes the phosphorylated Y701 in Stat1α and Stat1β.



Flow cytometric analysis of Stat1 (pY701). *U-937 cells (Human histiocytic lymphoma; ATCC CRL-1593.2) were either left unstimulated (unshaded) or stimulated (shaded) with 1000 U/mL recombinant human IFN-γ (Cat. No. 554617) for 15 minutes at 37°C. Cells were fixed with BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37°C and then permeabilized by adding BD Phosflow™ Perm Buffer III (Cat. No. 558050 for 30 minutes on ice. Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656) and stained with the Alexa Fluor® 647 mouse anti-Stat1 (pY701) antibody. Cells were analyzed on a BD FACSCalibur™ flow cytometery instrument. For intracellular staining of human whole blood, BD Phosflow™ Lyse/Fix buffer (Cat. No. 558049) may be used for fixation.*

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Investigators are encouraged to reference http://www.bdbiosciences.com/research/ics/resources/index.jsp for more information.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)
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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

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)

Fu XY, Zhang JJ. Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-fos gene promoter. *Cell.* 1993; 74(6):1135-1145. (Biology)

Perez OD, Mitchell D, Campos R, Gao GJ, Li L, Nolan GP. Multiparameter analysis of intracellular phosphoepitopes in immunophenotyped cell populations by flow cytometry. *Curr Protoc Cytom.* 2005; :6.20.1-6.20.22. (Clone-specific: Flow cytometry)

Suni MA, Maino VC. Flow cytometric analysis of cell signaling proteins. *Methods Mol Biol.* 2011; 717:155-169. (Clone-specific)

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)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**