Technical Data Sheet

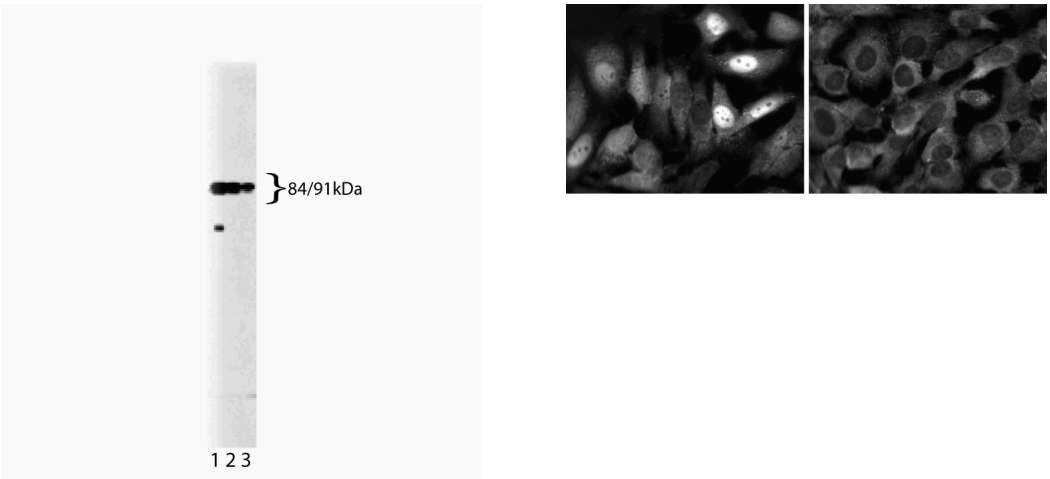
Purified Mouse Anti-Stat1

Product Information

Material Number:	**610186**
Alternate Name:	C-Terminus
Size:	150 µg
Concentration:	250 µg/ml
Clone:	42/Stat1
Immunogen:	Human Stat1 aa. 592-731
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	91/84 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The Stat proteins function as both cytoplasmic signal transducers and activators of transcription. The Stat91/84 (the two proteins are the result of alternate splicing-Stat91 has an additional 38 C-terminal amino acids) and Stat113 were the first identified members of this protein family. These three polypeptides contain both SH2 and SH3 domains and have also been described as members of the ISGF3 (interferon-stimulated gene factor 3) complex. With the discovery of additional members of the Stat family (Stats3 & 4), the nomenclature has been revised to indicate the Stat family members in the order of their discovery. Stat 91, 84, and 113 have become Stat1α, Stat1β, and Stat2, respectively. Stat1α is present in a higher concentration than Stat1β in most cell types. In response to IFNα treatment, Stat1α, Stat1β, and Stat2 become tyrosine-phosphorylated and migrate to the nucleus where they join a 48kDa DNA binding protein and subsequently direct the transcription at IFNα responsive elements. In IFN-γ treated cells, Stat1α (but not Stat2) becomes phosphorylated and forms a dimer. It then enters the nucleus and binds to the IFN-γ activated site (GAS) element in order to direct IFN-γ activated transcription.



Left Figure: Western blot analysis of Stat1 on a A431 lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the anti-Stat1 antibody.* ***Right Figure: Immunofluorescent staining of HeLa (ATCC CCL-2) cells.*** *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were either mock treated (media, left) or exposed to IFN-γ (100 ng/ml, right) for 15 minutes. After treatment, cells were stained using the alcohol perm protocol and the anti-Stat1 antibody. The second step reagent was Alexa-Fluor® 555 anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells. The recommended permeabilization agent is Perm Buffer III (see recommended assay procedure).*

Preparation and Storage

Store undiluted at -20°C.

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

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunoprecipitation	Reported

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
611447	A431 Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
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.
6. Triton is a trademark of the Dow Chemical Company.

References

Akira S, Nishio Y, Inoue M, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell.* 1994; 77(1):63-71. (Biology)

Ali MS, Sayeski PP, Bernstein KE. Jak2 acts as both a STAT1 kinase and as a molecular bridge linking STAT1 to the angiotensin II AT1 receptor. *J Biol Chem.* 2000; 275(20):15586-15593. (Clone-specific: Immunoprecipitation, Western blot)

Rayanade RJ, Ndubuisi MI, Etlinger JD, Sehgal PB. Regulation of IL-6 signaling by p53: STAT3- and STAT5-masking in p53-Val135-containing human hepatoma Hep3B cell lines. *J Immunol.* 1998; 161(1):325-334. (Clone-specific: Immunofluorescence, Western blot)

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Zhou YC, Waxman DJ. Cross-talk between janus kinase-signal transducer and activator of transcription (JAK-STAT) and peroxisome proliferator-activated receptor-alpha (PPARalpha) signaling pathways. Growth hormone inhibition of pparalpha transcriptional activity mediated. *J Biol Chem.* 1999; 274(5):2672-2681. (Clone-specific: Gel shift, Western blot)