abcam

Product datasheet

Anti-STAT1 antibody [EPR4407] ab109320

KO VALIDATED Recombinant RabMAb

16 References 8 Images

Overview

Product name	Anti-STAT1 antibody [EPR4407]
Description	Rabbit monoclonal [EPR4407] to STAT1
Host species	Rabbit
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A431, 293T, and MCF-7 cell lysates ICC/IF: MCF-7 cells Flow Cyt (intra): HeLa IHC-P: Human ovary carcinoma tissue. IP: MCF7. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR4407
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109320 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
Flow Cyt (Intra)		1/1000 - 1/10000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/250.
WB		1/10000 - 1/50000. Predicted molecular weight: 87 kDa.
IP		1/10 - 1/100.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

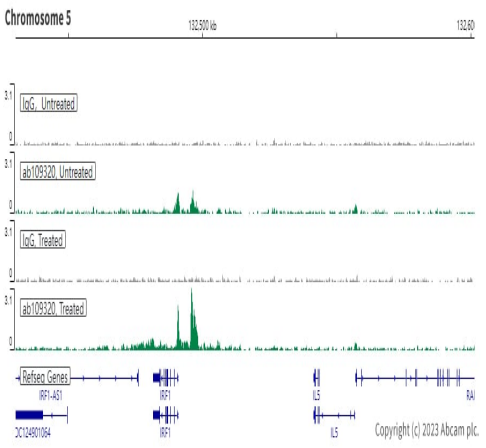
Target

Function Signal transducer and activator of transcription that mediates signaling by interferons (IFNs). Following type I IFN (IFN-alpha and IFN-beta) binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are activated, leading to tyrosine phosphorylation of STAT1 and STAT2. The phosphorylated STATs dimerize, associate with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor, that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the transcription of interferon stimulated genes, which drive the cell in an antiviral state. In response to type II IFN (IFN-gamma), STAT1 is tyrosine- and serine-phosphorylated. It then forms a homodimer termed IFN-gamma-activated factor (GAF), migrates into the nucleus and binds to the IFN gamma activated sequence (GAS) to drive the expression of the target genes, inducing a cellular antiviral state.

Involvement in disease Note=STAT1 deficiency results in impaired immune response leading to severe mycobacterial and viral diseases. In the case of complete deficiency, patients can die of viral disease. Defects in STAT1 are a cause of mendelian susceptibility to mycobacterial disease (MSMD) [MIM:209950]; also known as familial disseminated atypical mycobacterial infection. This rare condition confers predisposition to illness caused by moderately virulent mycobacterial species, such as Bacillus Calmette-Guerin (BCG) vaccine and environmental non-tuberculous mycobacteria, and by the more virulent Mycobacterium tuberculosis. Other microorganisms rarely cause severe clinical disease in individuals with susceptibility to mycobacterial infections, with the exception of Salmonella which infects less than 50% of these individuals. The pathogenic mechanism underlying MSMD is the impairment of interferon-gamma mediated immunity whose severity determines the clinical outcome. Some patients die of overwhelming mycobacterial

	disease with lepromatous-like lesions in early childhood, whereas others develop, later in life, disseminated but curable infections with tuberculoid granulomas. MSMD is a genetically heterogeneous disease with autosomal recessive, autosomal dominant or X-linked inheritance.
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Phosphorylated on tyrosine and serine residues in response to IFN-alpha, IFN-gamma, PDGF and EGF. Phosphorylation on Tyr-701 (lacking in beta form) by JAK promotes dimerization and subsequent translocation to the nucleus. Phosphorylation on Ser-727 by several kinases including MAPK14, ERK1/2 and CAMKII on IFN-gamma stimulation, regulates STAT1 transcriptional activity. Phosphorylation on Ser-727 promotes sumoylation though increasing interaction with PIAS. Phosphorylation on Ser-727 by PKCdelta induces apoptosis in response to DNA-damaging agents. Sumoylated by SUMO1, SUMO2 and SUMO3. Sumoylation is enhanced by IFN-gamma-induced phosphorylation on Ser-727, and by interaction with PIAS proteins. Enhances the transactivation activity. ISGylated.
Cellular localization	Cytoplasm. Nucleus. Translocated into the nucleus in response to IFN-gamma-induced tyrosine phosphorylation and dimerization.

Images

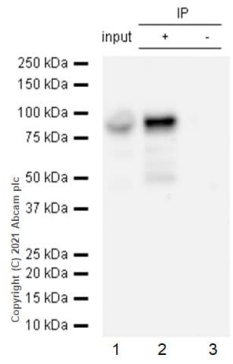


ChIC/CUT&RUN sequencing - Anti-STAT1 antibody [EPR4407] (ab109320)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells treated with IFN gamma (50ng/ml 1h) and 5µg of ab109320 [EPR4407]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Immunoprecipitation - Anti-STAT1 antibody [EPR4407] (ab109320)

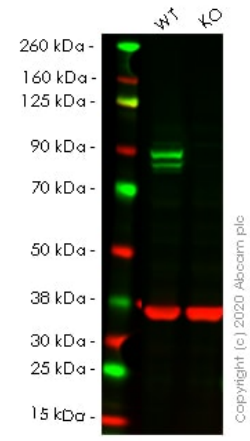
STAT1 was immunoprecipitated from 0.35 mg MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10 µg with 109320 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab109320 IP in MCF7 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab109320 in MCF7 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-STAT1 antibody [EPR4407] (ab109320)

All lanes : Anti-STAT1 antibody [EPR4407] (ab109320) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

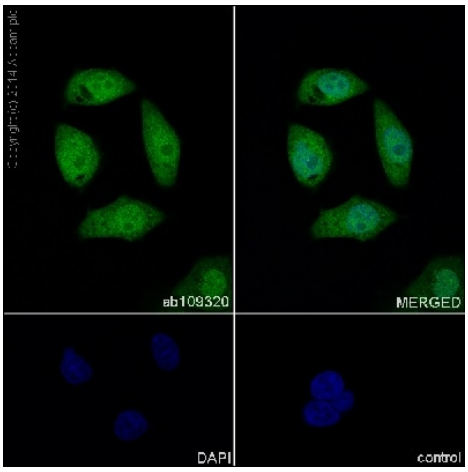
Performed under reducing conditions.

Predicted band size: 87 kDa
Observed band size: 87 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109320 observed at 87 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109320 was shown to react with STAT1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line

ab255346 (knockout cell lysate **ab263837**) was used. Wild-type HeLa and STAT1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109320 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

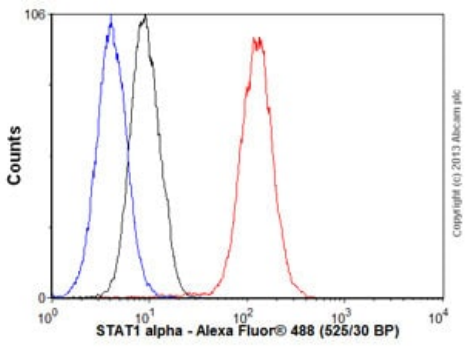


Immunocytochemistry/ Immunofluorescence - Anti-STAT1 antibody [EPR4407] (ab109320)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF-7(Human breast adenocarcinoma cell line) cell line labeling STAT1 with ab109320 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

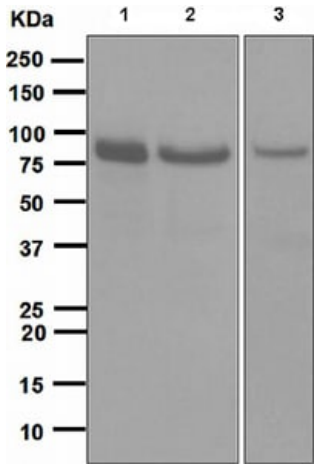
Confocal image showing nuclear and cytoplasmic staining on MCF7 cells

The nuclear counterstain is DAPI (blue).



Flow Cytometry (Intracellular) - Anti-STAT1 antibody [EPR4407] (ab109320)

Overlay histogram showing HeLa cells stained with ab109320 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109320, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



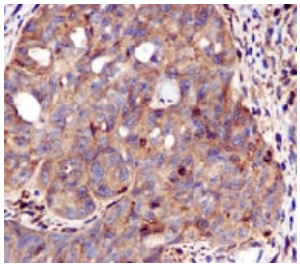
Western blot - Anti-STAT1 antibody [EPR4407] (ab109320)

All lanes : Anti-STAT1 antibody [EPR4407] (ab109320) at 1/10000 dilution

Lane 1 : 293T cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 87 kDa
Observed band size: 90 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT1 antibody [EPR4407] (ab109320)

ab109320 at 1/100 dilution staining STAT1 in Human ovary carcinoma by Immunohistochemistry, Paraffin-embedded tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-STAT1 antibody [EPR4407] (ab109320)

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