abcam

Product datasheet

Anti-STAT3 antibody [EPR787Y] ab68153

KO VALIDATED Recombinant RabMAb

10 Abreviews 285 References 8 Images

Overview

Product name	Anti-STAT3 antibody [EPR787Y]
Description	Rabbit monoclonal [EPR787Y] to STAT3
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, ICC/IF, WB, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Rat and mouse heart tissue lysates; Human, mouse, and rat brain tissue lysates; Rat kidney tissue lysate; HeLa, HAP1, A431, HaCaT, NIH/3T3, C2C12, and Raji cell lysates. IHC-P: Human brain tissue. ICC/IF: HeLa cells. Flow Cyt (intra): Raji cells. ChIC/CUT&RUN seq: HepG2 cell
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**.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR787Y

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab68153 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
Flow Cyt (Intra)		1/30 - 1/50.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF	★★★★☆ (1)	1/200. **For unpurified use at 1/140.**
WB	★★★★★ (7)	1/1000 - 1/2000. Detects a band of approximately 75, 88 kDa (predicted molecular weight: 88 kDa).
IHC-P	★★★☆☆ (1)	1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **For unpurified use at 1/140.**

Application notes Is unsuitable for IP.

Target

Function Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transctivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.

Tissue specificity Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.

Involvement in disease Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant
Autoimmune disease, multisystem, infantile-onset

Sequence similarities Belongs to the transcription factor STAT family.
Contains 1 SH2 domain.

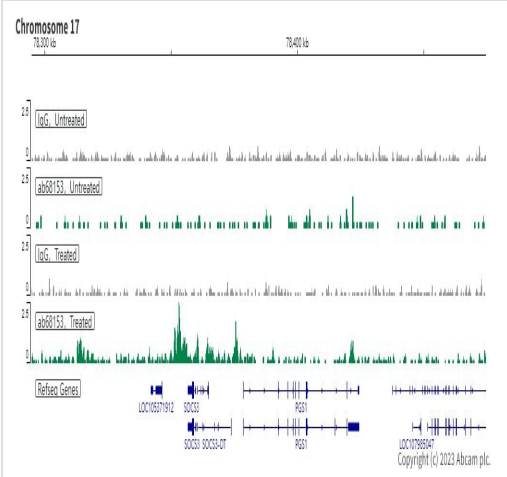
Post-translational modifications Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF, KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation

is important for the formation of stable DNA-binding STAT3 homodimers and maximal transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

Cellular localization

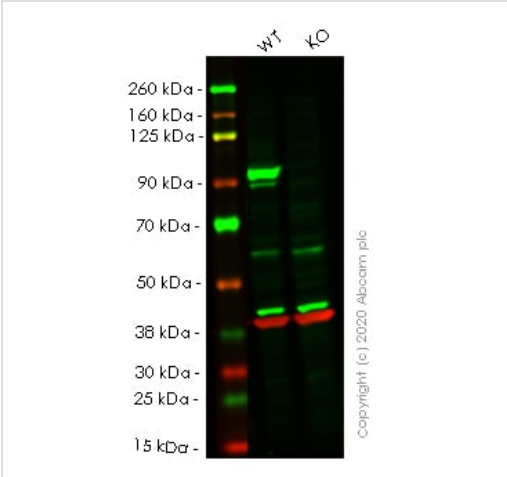
Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

Images



ChIC/CUT&RUN sequencing - Anti-STAT3 antibody [EPR787Y] (ab68153)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL, 2.5 x 10^5 HepG2 cells (starved overnight and treated with 100ng/ml IL-6 for 30min) and 5 µg of ab68153 [EPR787Y]. 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.



Western blot - Anti-STAT3 antibody [EPR787Y] (ab68153)

All lanes : Anti-STAT3 antibody [EPR787Y] (ab68153) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT3 knockout HeLa cell lysate

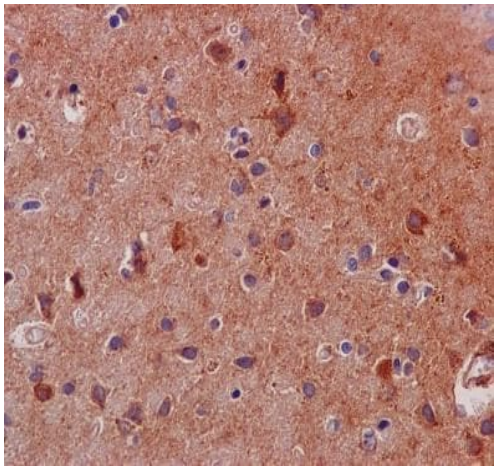
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 92 kDa

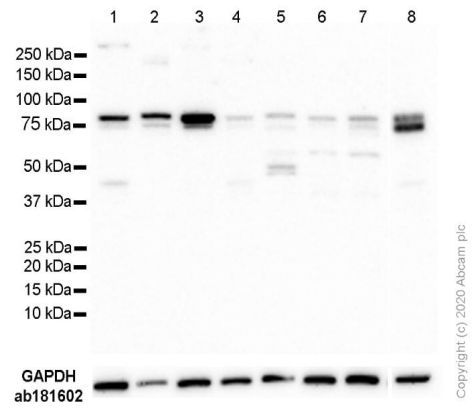
Lanes 1- 2: Merged signal (red and green). Green - ab68153 observed at 92 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab68153 was shown to react with STAT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255436** (knockout cell lysate **ab263797**) was used. Wild-type HeLa and STAT3 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. ab68153 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 antibody [EPR787Y] (ab68153)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue sections labelling STAT3 with purified ab68153 at 1/200. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.



Western blot - Anti-STAT3 antibody [EPR787Y] (ab68153)

All lanes : Anti-STAT3 antibody [EPR787Y] (ab68153) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HaCaT (Human skin keratinocyte) whole cell lysate
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 4 : C2C12 (Mouse myoblast cell line) whole cell lysate
Lane 5 : Human brain lysate
Lane 6 : Mouse brain lysate
Lane 7 : Rat brain lysate
Lane 8 : Rat kidney lysate

Lysates/proteins at 20 µg per lane.

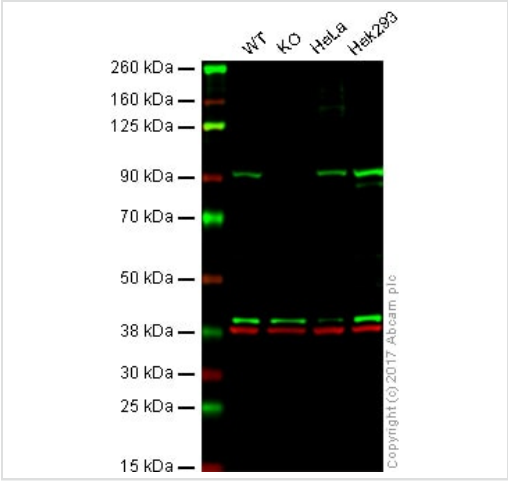
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 88 kDa
Observed band size: 92 kDa

Exposure time: 90 seconds

Blocking/Diluting buffer: 5% NFDM/TBST.

Rabbit monoclonal [EPR16891] to GAPDH (**ab181602**) used as loading control.



Western blot - Anti-STAT3 antibody [EPR787Y] (ab68153)

All lanes : Anti-STAT3 antibody [EPR787Y] (ab68153) at 1/500 dilution

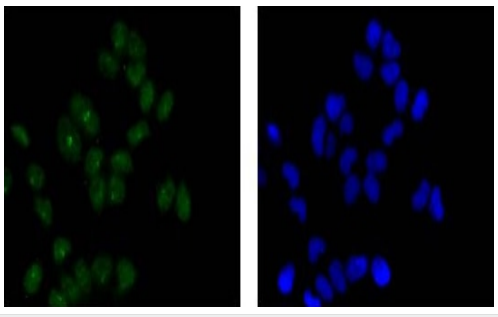
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : STAT3 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 88 kDa

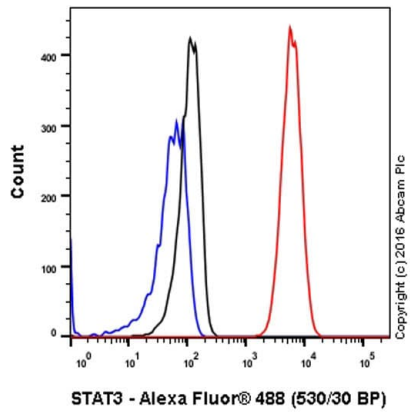
Lanes 1 - 4: Merged signal (red and green). Green - ab68153 observed at 92 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Ab68153 detected the expected band for STAT3 in wild-type cells along with additional cross-reactive bands. The band was not seen in STAT3 knockout HAP1 cells. Wild-type and STAT3 knockout samples were subjected to SDS-PAGE. Ab68153 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-STAT3 antibody [EPR787Y] (ab68153)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling STAT3 (green) with purified ab68153 at 1/200. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

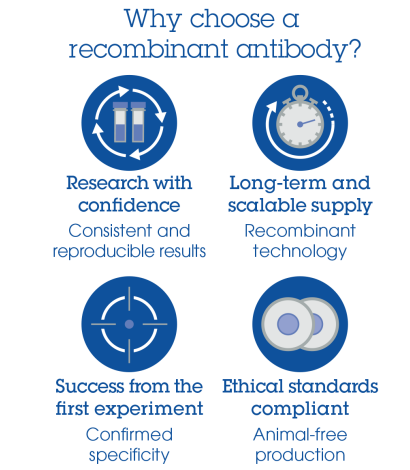


Flow Cytometry (Intracellular) - Anti-STAT3 antibody [EPR787Y] (ab68153)

ab68153 stainingSTAT3in the human cell line HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/30. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Anti-STAT3 antibody [EPR787Y] (ab68153)

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