abcam

Product datasheet

Anti-STAT3 antibody [9D8] ab119352

★★★★☆ 11 Abreviews 181 References 11 Images

Overview

Product name Anti-STAT3 antibody [9D8]

Description Mouse monoclonal [9D8] to STAT3

Host species Mouse

Specificity Detects STAT3 from Human, murine, monkey and rat samples.

Tested applications Suitable for: Flow Cyt, ICC/IF, WB, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human, African green monkey

Immunogen Recombinant fragment, corresponding to amino acids 665-770 of Human STAT3.

Positive control HepG2, HeLa, PC-12, A549, 293T, Jurkat, A431, U2OS, MCF7, A549, K562, NIH3T3, C2C12,

and NRK whole cell lysates; U2OS lysate from STAT3 SMART pool siRNA transfected; Mouse lung tissue lysate; HeLa cells; Human pancreas, brain tumour, colon cancer and stomach cancer

tissues Flow Cyt: HeLa cells.

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 Preservative: 0.05% Sodium azide

Constituents: 99% PBS, 0.1% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number 9D8

Isotype IgG

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Applications

The Abpromise quarantee

Our Abpromise quarantee covers the use of ab119352 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		Use 0.1µg for 10 ⁶ cells.
ICC/IF		1/100.
WB	****(2)	1/5000. Detects a band of approximately 88 kDa (predicted molecular weight: 88 kDa).
IP		Use at 2 µg/mg of lysate.
IHC-P	★★★★ ★ (5)	1/1600. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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

 $\label{prop:continuous} \mbox{Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant}$

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

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

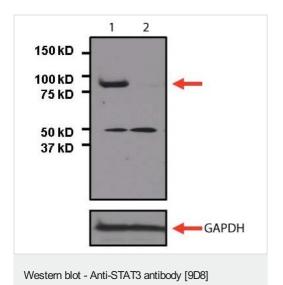
Cellular localization

residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

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

(ab119352)



All lanes : Anti-STAT3 antibody [9D8] (ab119352) at 1/1000 dilution

Lane 1: U2OS lysate from non-targeting control

Lane 2: U2OS lysate from STAT3 SMART pool siRNA transfected

Lysates/proteins at 25 µg per lane.

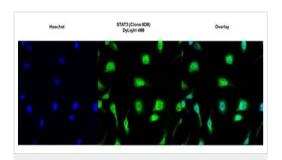
Secondary

All lanes: goat anti-mouse-HRP at 1/20000 dilution

Developed using the ECL technique.

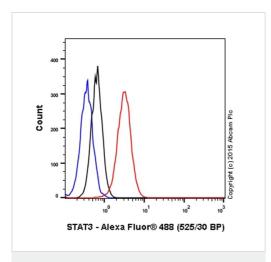
Predicted band size: 88 kDa

4-20% Tris-HCI polyacrylamide gel

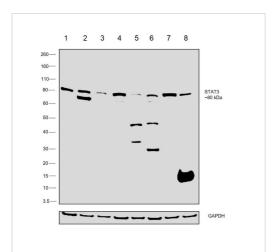


Immunocytochemistry/ Immunofluorescence - Anti-STAT3 antibody [9D8] (ab119352)

Immunofluorescent analysis of STAT3 using <u>ab119352</u> at 1/100 dilution (shown in green) in HeLa cells (formalin fixed cells permeabilized with 0.1% Triton X-100) using a DyLight 488 goatanti-mouse secondary antibody (<u>ab96879</u>) at 1/400 dilution. Nuclei (blue) were stained with Hoechst 33342 dye.



Flow Cytometry - Anti-STAT3 antibody [9D8] (ab119352)



Western blot - Anti-STAT3 antibody [9D8] (ab119352)

Overlay histogram showing HeLa cells stained with ab119352 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween 20 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 (ab119352, 0.1µg/1x10 6 cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse lgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG (ab37355, 0.1µg/1x106 cells) used under the same conditions. Unlabelled

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

sample (blue line) was also used as a control.

All lanes : Anti-STAT3 antibody [9D8] (ab119352) at 1/5000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2 : A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3: Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 4 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 5: PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

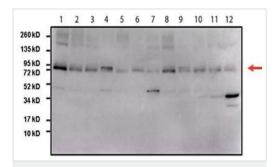
Lane 6 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 7: A549 (Human lung carcinoma cell line) whole cell lysate

Lane 8: Mouse lung tissue lysate

Lysates/proteins at 30 µg per lane.

Predicted band size: 88 kDa **Observed band size:** 80 kDa



Western blot - Anti-STAT3 antibody [9D8] (ab119352)

All lanes : Anti-STAT3 antibody [9D8] (ab119352) at 1/5000 dilution

Lane 1: HepG2 cell lysate

Lane 2: 293T cell lysate

Lane 3: Jurkat cell lysate

Lane 4: A431 cell lysate

Lane 5: U2OS cell lysate

Lane 6 : MCF7 cell lysate

Lane 7: A549 cell lysate

Lane 8 : K562 cell lysate

Lane 9 : COS7 cell lysate

Lane 10: NIH3T3 cell lysate

Lane 11: C2C12 cell lysate

Lane 12: NRK cell lysate

Lysates/proteins at 25 µg per lane.

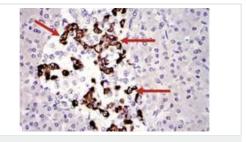
Secondary

All lanes: goat anti-mouse-HRP at 1/20000 dilution

Developed using the ECL technique.

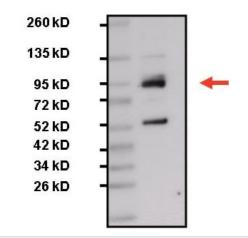
Predicted band size: 88 kDa

4-20% Tris-HCl polyacrylamide gel



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT3 antibody [9D8] (ab119352)

ab119352, at 1/1600 dilution, staining STAT3 in Human pancreas tissue by Immunohistochemistry. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate. Tissues were counterstained with hematoxylin.



Western blot - Anti-STAT3 antibody [9D8] (ab119352)

4-20% Tris-HCl polyacrylamide gel

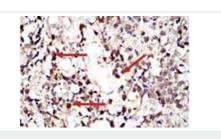
goat anti-mouse-HRP at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 88 kDa

total lysate at 25 µg

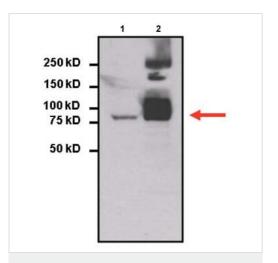
Secondary



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT3 antibody [9D8] (ab119352)

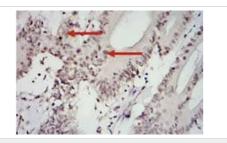
ab119352, at 1/1600 dilution, staining STAT3 in Human brain tumor tissue by Immunohistochemistry. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate. Tissues were counterstained with hematoxylin.

Anti-STAT3 antibody [9D8] (ab119352) at 1/1000 dilution + HepG2



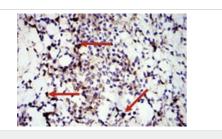
Immunoprecipitation - Anti-STAT3 antibody [9D8] (ab119352)

Immunoprecipitation of STAT3 was performed on HepG2 cells. The antigen:antibody complex was formed by incubating 750µg whole cell lysate with 2µg of ab119352 overnight at 4°C. The immunecomplex was captured, washed extensively and proteins eluted with 5X Reducing Sample Loading Dye. Samples were resolved on a 4-20% Tris-HCI polyacrylamide gel. Proteins were transferred to PVDF membrane and blocked with 5% Milk/TBS-0.1% Tween for at least 1 hour. Membranes were then probed with ab119352 at a dilution of 1/5000 overnight at 4°C. Membranes were washed in TBST and probed with IP detection reagent at a dilution of 1/2000 for at least one hour. Membranes were washed and chemiluminescent detection was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT3 antibody [9D8] (ab119352)

ab119352, at 1/1600 dilution, staining STAT3 in Human colon cancer tissue by Immunohistochemistry. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate. Tissues were counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT3 antibody [9D8] (ab119352)

<u>ab119352</u>, at 1/1600 dilution, staining STAT3 in Human stomach cancer tissue by Immunohistochemistry. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate. Tissues were counterstained with hematoxylin.

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