abcam

Product datasheet

Anti-STAT3 (phospho S727) antibody [E121-31] ab32143

Recombinant RabMAb

★★★★★ 7 Abreviews 73 References 13 Images

Overview

Product name	Anti-STAT3 (phospho S727) antibody [E121-31]
Description	Rabbit monoclonal [E121-31] to STAT3 (phospho S727)
Host species	Rabbit
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, ICC/IF, WB, Dot blot, IP, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Horse, Cow, Macaque monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431 cell lysate, C6 treated with epidermal growth factor. IP: HeLa cells ICC/IF: A431 cells IHC-P: human astrocytoma, rat cerebral cortex, mouse liver, and brain astrocytoma tissues 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**. 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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	E121-31
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32143 in the following tested applications.

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

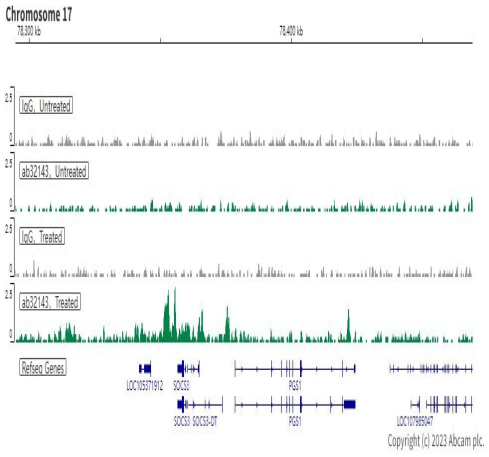
Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		1/500. **For unpurified, use 1/100.**
WB	★★★★★ **(1)**	1/1000 - 1/10000. Detects a band of approximately 98 kDa (predicted molecular weight: 88 kDa). Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.
Dot blot		1/1000.
IP		1/60.
IHC-P	★★★★★ **(5)**	1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified, use 1/50.**

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

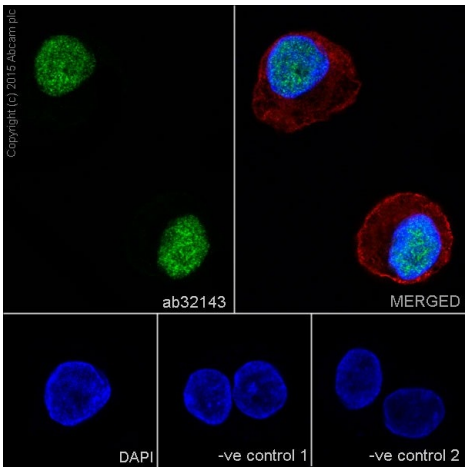
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through 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



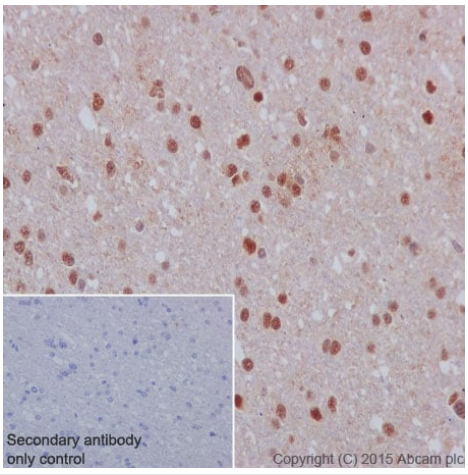
ChIC/CUT&RUN sequencing - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

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 ab32143 [E121-31]. 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.



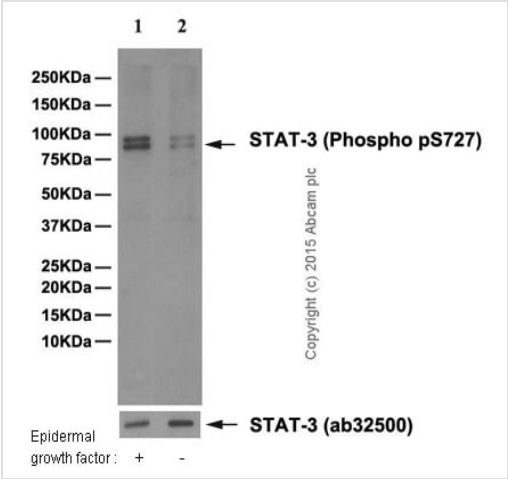
Immunocytochemistry/ Immunofluorescence - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Purified ab32143 staining STAT3 (phospho S727) in A431 cells by Immunocytochemistry/ Immunofluorescence. 4% PFA-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma) cells labelled with ab32143 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Confocal image showing nuclear staining on A431 cell line. The red staining is **ab7291** anti-Tubulin (mouse mAb), followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Immunohistochemical staining of paraffin embedded human astrocytoma with purified ab32143 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

All lanes : Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143) at 1/5000 dilution (purified)

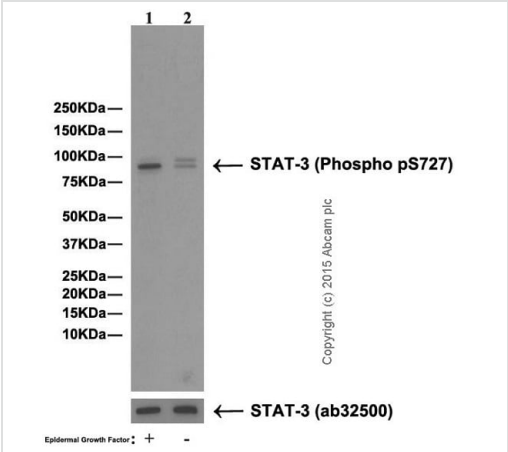
Lane 1 : C6 treated with epidermal growth factor
Lane 2 : untreated C6 whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

Predicted band size: 88 kDa
Observed band size: 98 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



Western blot - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

All lanes : purified

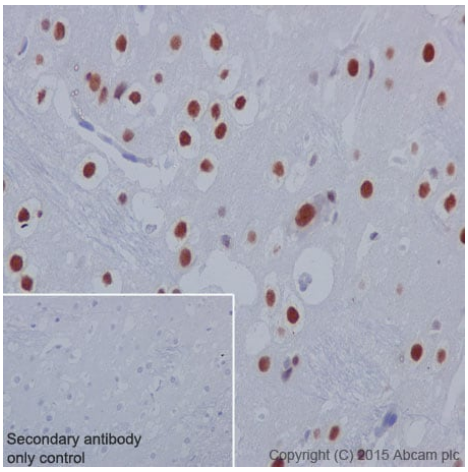
Lane 1 : A431 treated with epidermal growth factor
Lane 2 : untreated A431 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

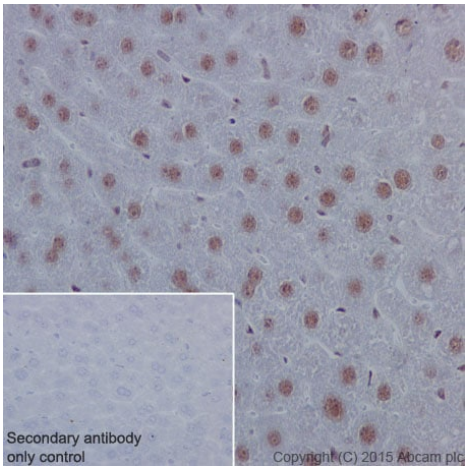
Predicted band size: 88 kDa
Observed band size: 98 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



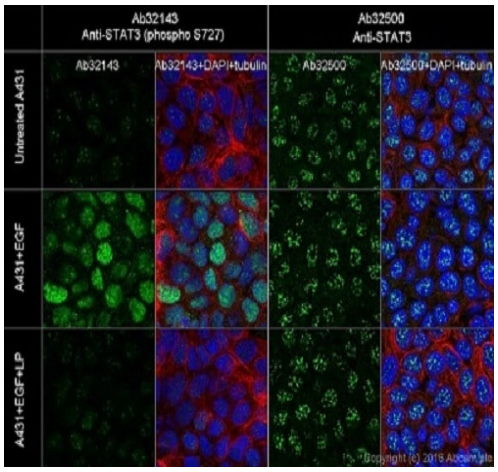
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Immunohistochemical staining of paraffin embedded rat cerebral cortex with purified ab32143 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Immunohistochemical staining of paraffin embedded mouse liver with purified ab32143 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

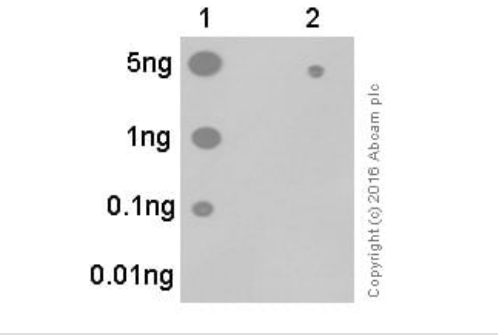


Immunocytochemistry/ Immunofluorescence - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Immunocytochemical/Immunofluorescence analysis of untreated, EGF treated and EGF + LP treated A431 cells labelling STAT3 (phospho S727) with ab32143 (left) and STAT3 with **ab32500** (right) both at a dilution of 1/500.

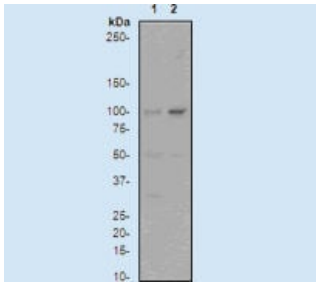
Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) (1/1000) was used as the secondary antibody (green). DAPI (blue) was used as the nuclear counterstain. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (1/200) was used as a counterstain (red).

The green staining was increased and translocated from the cytoplasm into the nucleus in the EGF (**ab9697** 100ng/ml, 10min) treated A431 cells when compared with A431 cells without treatment. After LP treatment, the green signal was decreased. For the pan antibody, there was no great difference after EGF (100ng/ml, 10min) or EGF (100ng/ml, 10min) + LP treatment.



Dot Blot - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

Dot Blot analysis of Lane 1: STAT3 (pS727) phospho peptide and Lane 2: STAT3 non-phospho peptide labeling STAT3 (phospho S727) with ab32143 at 1/1000 dilution (0.009 µg/ml). 5% NFDM /TBST was used as the diluting and blocking buffer and concentration. **ab97051**, Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as the secondary antibody at 1/100,000 dilution. Exposure time: 10 seconds.

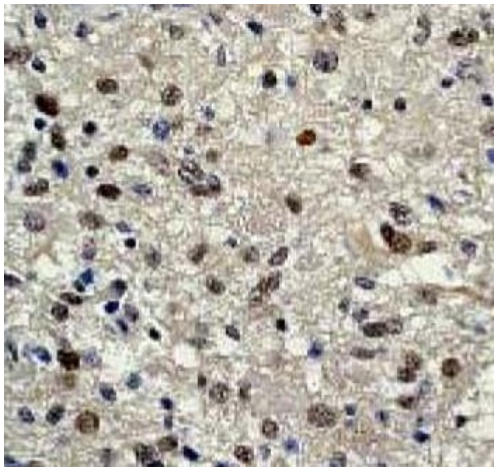


Western blot - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

All lanes : Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143) at 1/1000 dilution (unpurified)

Lane 1 : A431 cell lysate
Lane 2 : A431 + EGF cell lysate

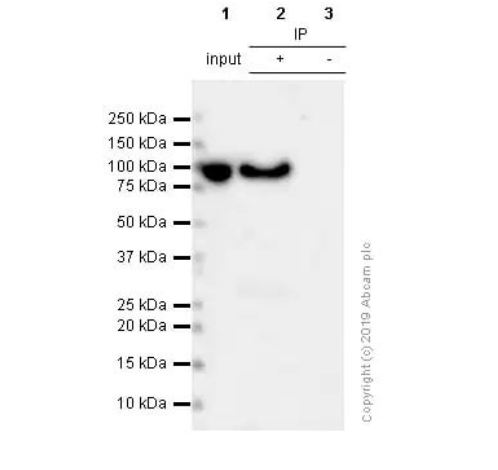
Predicted band size: 88 kDa
Observed band size: 98 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

IHC-P analysis of brain astrocytoma using unpurified ab32143 at 1/50 dilution.

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



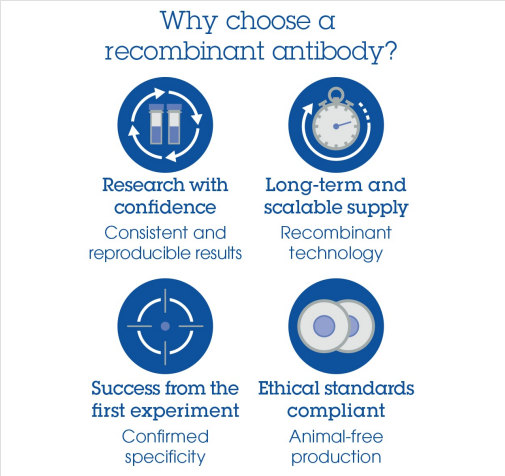
Immunoprecipitation - Anti-STAT3 (phospho S727) antibody [E121-31] (ab32143)

ab32143 (purified) at 1/60 dilution (2.594 µg/ml) immunoprecipitating STAT3 in HeLa whole cell lysate.

Lane 1 (input): HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32143 & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32143 in HeLa whole cell lysate

For western blotting, ab32143 at 1/500 and VeriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM /TBST.



Anti-STAT3 (phospho S727) antibody [E121-31]

(ab32143)

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