# abcam

# Product datasheet

# Anti-STAT3 antibody [E121-21] - BSA and Azide free ab171361



Recombinant

RabMAb

★★★★☆ 1 Abreviews 1 References 6 Images

#### Overview

Product name Anti-STAT3 antibody [E121-21] - BSA and Azide free

**Description** Rabbit monoclonal [E121-21] to STAT3 - BSA and Azide free

Host species Rabbit

**Specificity** This antibody only detects Stat3 without phosphorylation on Serine 727. It does not detect S727-

phosphorylated Stat3.

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP

Unsuitable for: IHC-P

Species reactivity Reacts with: Human

Predicted to work with: Horse, Cow

Immunogen Synthetic peptide corresponding to Human STAT3. A synthetic peptide corresponding to

residues surrounding Ser727 of human Stat3.

**General notes** ab171361 is the carrier-free version of <u>ab32500</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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

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For more information see here.

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number E121-21

**Isotype** IgG

#### **Applications**

## The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab171361 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)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 92 kDa (predicted molecular weight: 88 kDa).
IP		Use at an assay dependent concentration.

Application notes

Is unsuitable for IHC-P.

#### **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**



Western blot - Anti-STAT3 antibody [E121-21] - BSA and Azide free (ab171361)

**All lanes:** Anti-STAT3 antibody [E121-21] (ab32500) at 1/1000 dilution (Purified)

Lane 1: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate

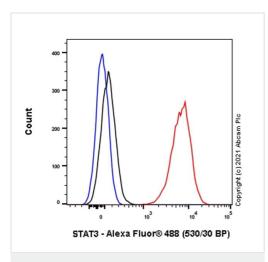
Lane 2: HaCaT (Human skin keratinocyte) whole cell lysate

Secondary

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

dilution

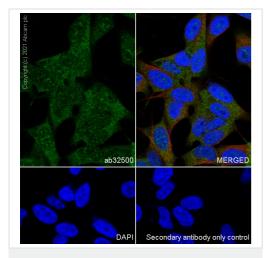
Predicted band size: 88 kDa



Flow Cytometry (Intracellular) - Anti-STAT3 antibody [E121-21] - BSA and Azide free (ab171361)

This data was developed using ab171361, the same antibody clone in a different buffer formulation.

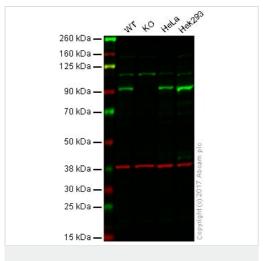
Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labelling STAT3 with Purified ab171361 at 1:20 dilution (5 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, **ab150081**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-STAT3 antibody [E121-21] - BSA and Azide free (ab171361)

This data was developed using ab171361, the same antibody clone in a different buffer formulation.

Immunocytochemistry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells labeling STAT3 with Purified ab171361 at 1:50 dilution (2.1  $\mu$ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2  $\mu$ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-STAT3 antibody [E121-21] - BSA and Azide free (ab171361)

This WB data was generated using the same anti-STAT3 antibody clone, E121-21, in a different buffer formulation (cat# **ab32500**).

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

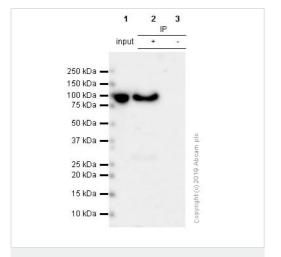
Lane 2: STAT3 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: HEK293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab32500</u> observed at 92 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

Ab32500 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. Ab32500 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.



Immunoprecipitation - Anti-STAT3 antibody [E121-21] - BSA and Azide free (ab171361)

**ab32500** (purified) at 1/500 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

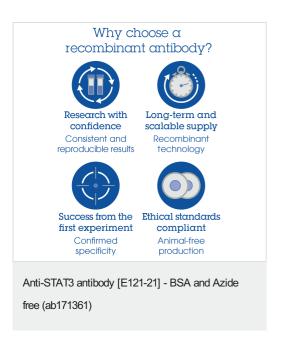
Lane 2 (+): ab32500 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG ( $\underline{ab172730}$ ) instead of  $\underline{ab32500}$  in HeLa whole cell lysate

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

Blocking and diluting buffer: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32500</u>).



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