# abcam

# Product datasheet

# Anti-STAT5a antibody [E289] - BSA and Azide free ab213219



# 7 References 11 Images

#### Overview

Product name Anti-STAT5a antibody [E289] - BSA and Azide free

**Description** Rabbit monoclonal [E289] to STAT5a - BSA and Azide free

Host species Rabbit

**Specificity** The antibody recognises Stat5a. It does not cross-react with other Stat family members. The

mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

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

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A431 cell lysate. IHC-P: Human squamous lung carcinoma. ICC/IF: Jurkat cells. Flow Cyt

(intra): Jurkat cells.

**General notes** ab213219 is the carrier-free version of <u>ab32043</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

1

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 pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

**Clonality** Monoclonal

Clone number E289

**Isotype** IgG

### **Applications**

#### The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab213219 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
WB		Use at an assay dependent concentration. Detects a band of approximately 92 kDa (predicted molecular weight: 91 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

# **Target**

**Function** Carries out a dual function: signal transduction and activation of transcription. Binds to the GAS

element and activates PRL-induced transcription.

**Sequence similarities**Belongs to the transcription factor STAT family.

Contains 1 SH2 domain.

Post-translational Tyrosine phosphorylated in response to IL-2, IL-3, IL-7, IL-15, GM-CSF, growth hormone,

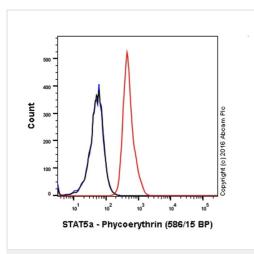
#### modifications

prolactin, erythropoietin and thrombopoietin. Tyrosine phosphorylation is required for DNA-binding activity and dimerization. Serine phosphorylation is also required for maximal transcriptional activity.

#### **Cellular localization**

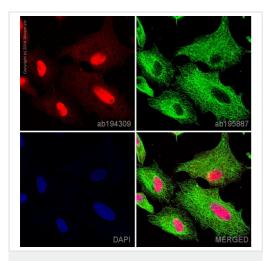
Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

#### **Images**



Flow Cytometry (Intracellular) - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219) Clone E289 (ab213219) has been successfully conjugated by Abcam. This image was generated using Anti-STAT5a antibody [E289] (PE). Please refer to **ab211686** for protocol details.

Overlay histogram showing A549 cells stained with <u>ab211686</u> (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (<u>ab211686</u>, 1/500 dilution) for 30 min at 22°C.lsotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (<u>ab209478</u>) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter. This antibody gave a positive signal in A549 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.

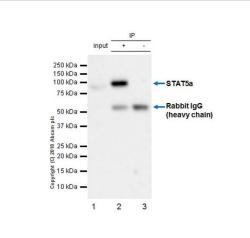


Immunocytochemistry/ Immunofluorescence - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

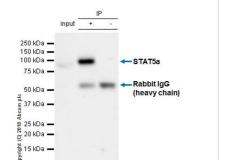
Clone E289 (ab213219) has been successfully conjugated by Abcam. This image was generated using Anti-STAT5a antibody [E289] (Alexa Fluor® 647). Please refer to **ab194309** for protocol details.

<u>ab194309</u> staining STAT5a in A549 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with <u>ab194309</u> at 1/100 Dilution(shown in red) and <u>ab195887</u>, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 488, shown in green) at 2μg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunoprecipitation - Anti-STAT5a antibody [E289] -BSA and Azide free (ab213219)



This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).

ab32043 (purified) at 1:20 dilution (0.6ug) immunoprecipitating in TF-1 whole cell lysate. TF-1 (Human Erythroleukemia erythroblast)

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32043

For western blotting, VeriBlot for IP Detection Reagent (HRP)

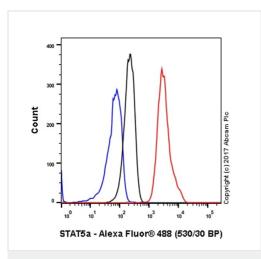
(ab131366), was used for detection at 1/1000 dilution

whole cell lysate 10ug

in TF-1 whole cell lysate

Lane 2 (+): ab32043 & TF-1 whole cell lysate

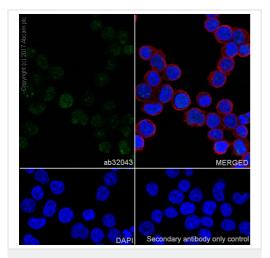
Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling STAT5a with purified ab32043 at 1/100 dilution (1 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

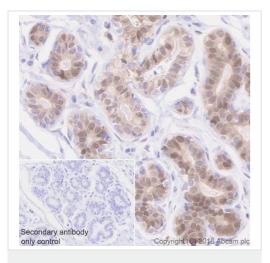
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).



Immunocytochemistry/ Immunofluorescence - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (human T cell leukemia T lymphocyte) cells labeling STAT5a with purified ab32043 at 1:100 (1.2 µg/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 µg/ml). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

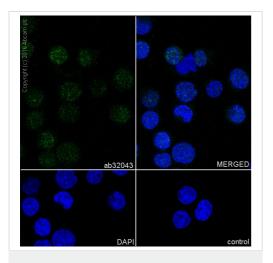
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast tissue sections labeling STAT5a with purified <a href="mailto:ab32043">ab32043</a> at 1:1000 dilution (0.12 µg/ml). Heat mediated antigen retrieval was performed using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use). PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).

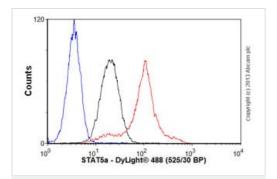


Immunocytochemistry/ Immunofluorescence - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling STAT5a with unpurified <a href="mailto:ab32043">ab32043</a> at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, an Alexa Fluor<sup>®</sup> 488-conjugated goat antirabbit lgG (1/1000) was used as the secondary antibody. Control: PBS only.

Nuclear counter stain: DAPI.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).

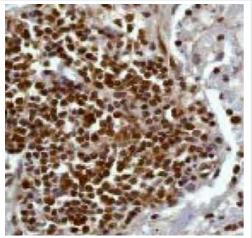


Flow Cytometry (Intracellular) - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Overlay histogram showing Jurkat cells stained with unpurified <a href="mailto:ab32043">ab32043</a> (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab32043, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat antirabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x106 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected

using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32043).

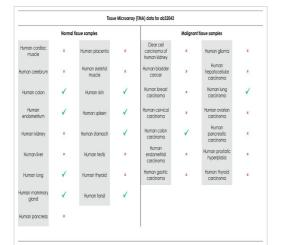


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a antibody [E289]

- BSA and Azide free (ab213219)

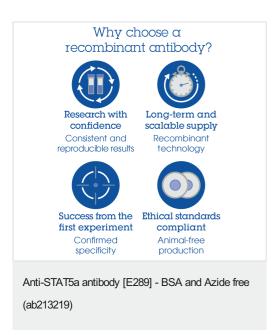
This IHC data was generated using the same anti-STAT5a antibody clone, E289, in a different buffer formulation (cat# <u>ab32043</u>).

Unpurified <u>ab32043</u> at 1/250 dilution, staining human squamous lung carcinoma by Immunohistochemistry, paraffin-embedded tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a antibody [E289] - BSA and Azide free (ab213219)

Tissue Microarrays stained for "Anti-STAT5a antibody [E289]" using " <u>ab32043</u>" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). The sections were incubated with <u>ab32043</u> at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

#### Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors