# abcam

#### Product datasheet

## Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free ab251306



#### 11 Images

#### Overview

**Product name** Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free

**Description** Rabbit monoclonal [EPR16668] to STAT5a+STAT5b - BSA and Azide free

**Host species** Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

**General notes** ab251306 is the carrier-free version of ab200341.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits 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® 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

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. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR16668

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab251306 in the following tested applications.

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

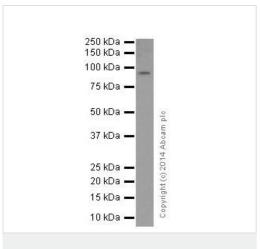
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 90 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

### **Target**

**Cellular localization** STAT5a: Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

STAT5b: Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

#### **Images**



Western blot - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

Anti-STAT5a + STAT5b antibody [EPR16668] (ab200341) at 1/200 dilution + Full length human Stat5a recombinant protein at 0.01  $\mu g$ 

#### Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

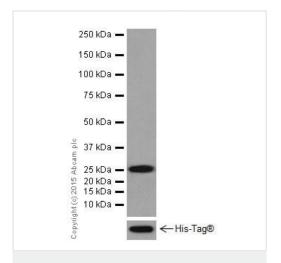
Predicted band size: 90 kDa Observed band size: 90 kDa

Exposure time: 3 minutes

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

<u>ab200341</u> show cross reactivity with STAT5a. Full length human Stat5a recombinant protein contains aa1-794 with MYC/DDDDK tag.



Western blot - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

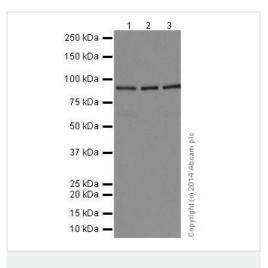
Anti-STAT5a + STAT5b antibody [EPR16668] (ab200341) at 1/10000 dilution + Recombinant protein fragment mouse Stat5b at 0.01  $\mu g$ 

#### **Secondary**

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 90 kDa

Exposure time: 3 minutes



Western blot - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

**All lanes**: Anti-STAT5a + STAT5b antibody [EPR16668] (ab200341) at 1/1000 dilution

**Lane 1 :** K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate

**Lane 2**: Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lane 3 : Daudi (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 90 kDa **Observed band size:** 90 kDa

Exposure time: 3 minutes

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

**All lanes**: Anti-STAT5a + STAT5b antibody [EPR16668] (ab200341) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lane 3: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lane 4 : Mouse heart lysate

Lane 5 : Rat heart lysate

Lysates/proteins at 10 µg per lane.

#### 1 2 3 4 5 250 kDa — 150 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 16 kDa — 17 kDa — 18 kDa — 19 kDa —

Western blot - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 90 kDa **Observed band size:** 90 kDa

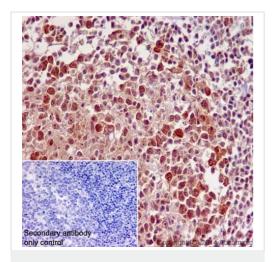
Exposure time: 3 minutes

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

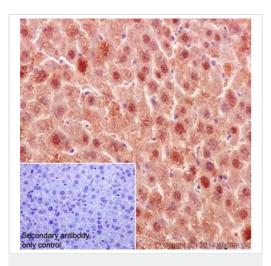
**Blocking and dilution buffer:** 5% NFDM/TBST.

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling STAT5b with <a href="mailto:ab200341">ab200341</a> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<a href="mailto:ab97051">ab97051</a>) secondary antibody at 1/500 dilution. Cytoplasm and nuclear staining on Human tonsil tissue is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<a href="mailto:ab97051">ab97051</a>) at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

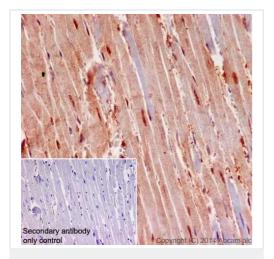


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)



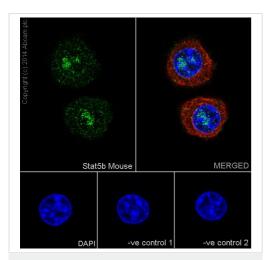
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

This data was developed using <a href="mailto:ab200341">ab200341</a>, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling STAT5b with <a href="mailto:ab200341">ab200341</a> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<a href="mailto:ab97051">ab97051</a>) secondary antibody at 1/500 dilution. Cytoplasm and nuclear staining on mouse liver tissue is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<a href="mailto:ab97051">ab97051</a>) at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.Immunohistochemical analysis of paraffin-embedded Rat cardiac muscle tissue labeling STAT5b with <u>ab200341</u> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Cytoplasm and nuclear staining on rat cardiac muscle tissue is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

Stat5b Mouse

MERGED

DAPI

-ve control 1

-ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306)

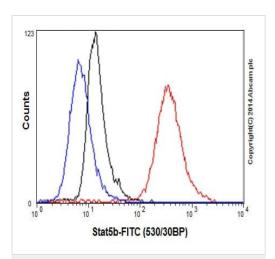
This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.lmmunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) cells labeling STAT5b with <u>ab200341</u> at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/500 dilution (green).Nuclear and cytoplasm staining on RAW 264.7 cell line is observed.The nuclear counter stain is DAPI (blue). Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).The negative controls are as follows:

-ve control 1: <u>ab200341</u> at 1/500 dilution followed by <u>ab150120</u>

-ve control 1: <u>ab200341</u> at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

This data was developed using <a href="mailto:ab200341">ab200341</a>, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C6 (Rat glial tumor cells) cells labeling STAT5b with <a href="mailto:ab200341">ab200341</a> at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/500 dilution (green). Nuclear and cytoplasm staining on C6 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with <a href="mailto:ab7291">ab7291</a> (anti-Tubulin mouse mAb) at 1/1000 dilution and <a href="mailto:ab150120">ab150120</a> (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/500 dilution (red). The negative controls are as follows:

-ve control 1: <u>ab200341</u> at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



Flow Cytometry (Intracellular) - Anti-STAT5a+STAT5b antibody [EPR16668] - BSA and Azide free (ab251306) This data was developed using <u>ab200341</u>, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed NIH/3T3 (Mouse embyro fibroblast cells) cells labeling STAT5b with <a href="mailto:ab200341">ab200341</a> at 1/80 dilution (red) compared with a rabbit monoclonal lgG isotype control (<a href="mailto:ab172730">ab172730</a>; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (FITC) at 1/150 dilution was used as the secondary antibody.



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