abcam

Product datasheet

Anti-STAT5a + STAT5b antibody [EPR16671-40] ab194898



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Overview

Product name Anti-STAT5a + STAT5b antibody [EPR16671-40]

Rabbit monoclonal [EPR16671-40] to STAT5a + STAT5b **Description**

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Human STAT5A full length recombinant protein; K562, Jurkat and Daudi cell lysates; Human

fetal heart and kidney lysates; mouse heart, kidney, spleen lysates; rat brain, heart, spleen lysates;

RAW 264.7, PC12, NIH/3T3 whole cell lysates. IHC-P: Human tonsils, mouse spleen and rat

spleen tissue. IF, Flow, IP: K562 cells

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**.

Properties

Form

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR16671-40

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab194898 in the following tested applications.

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

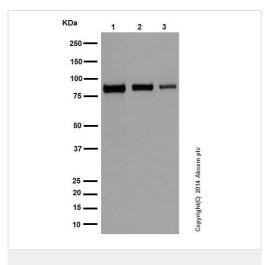
Application	Abreviews	Notes
Flow Cyt (Intra)		1/80. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P	* * * * * <u>(2)</u>	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	****(1)	1/2000. Detects a band of approximately 91 kDa (predicted molecular weight: 91 kDa).
ICC/IF		1/250.
IP		1/100.

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 [EPR16671-40] (ab194898)

All lanes : Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898) at 1/20000 dilution

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

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

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

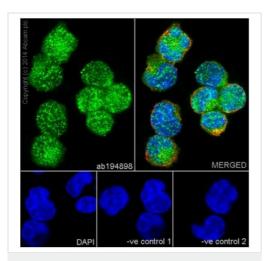
Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 91 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

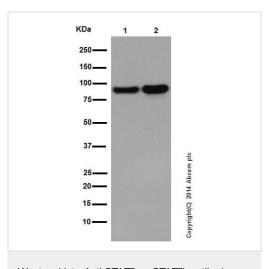


Immunocytochemistry/ Immunofluorescence - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling STAT5a + STAT5b with ab194898 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Confocal image showing both cytoplasmic and nuclear staining on K562 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor[®] 594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

- 1. ab194898 at 1/250 dilution followed by $\underline{ab150120}$ (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/500 dilution.
- 2. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor[®] 488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.



Western blot - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

All lanes : Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898) at 1/2000 dilution

Lane 1 : Human fetal heart lysates

Lane 2 : Human fetal kidney lysates

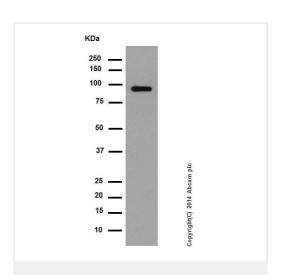
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Anti-Rabbit $\lg G$ (HRP), specific to the non-reduced form of $\lg G$ at 1/1000 dilution

Predicted band size: 91 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898) at 1/1000 dilution + Human STAT5A full length recombinant protein at $10~\mu g$

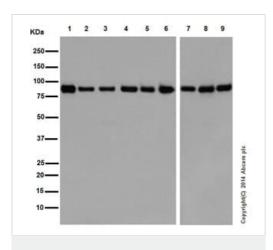
Secondary

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

Predicted band size: 91 kDa

Recombinant human C-terminal Myc/DDK tagged full length STAT5A from a commercial source.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

All lanes : Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898) at 1/2000 dilution

Lane 1: mouse heart lysates

Lane 2: mouse kidney lysates

Lane 3: mouse spleen lysates

Lane 4: rat brain lysates

Lane 5: rat heart lysates

Lane 6: rat spleen lysates

Lane 7: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysates

Lane 8: PC12 (Rat adrenal gland pheochromocytoma) whole cell

lysates

Lane 9: NIH3T3 (Mouse embyro fibroblast cells) whole cell lysates

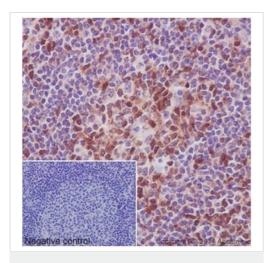
Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 91 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

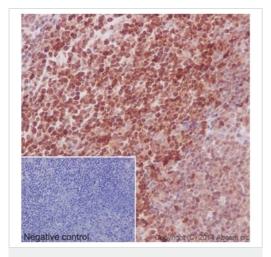


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling STAT5a + STAT5b using ab194898 at 1/2000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 was used as secondary. Counter stained with Hematoxylin. Inset image: negative control obtained using PBS instead of ab194898.

Nuclear and weak cytoplasmic staining on lymphocytes of human tonsil is observed.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

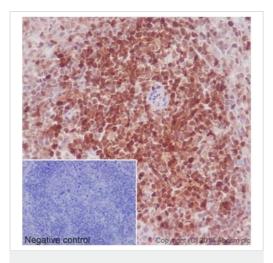


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling STAT5a + STAT5b using ab194898 at 1/2000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 was used as secondary. Counter stained with Hematoxylin. Inset image: negative control obtained using PBS instead of ab194898

Nuclear and weak cytoplasmic staining on lymphocytes of mouse spleen is observed.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

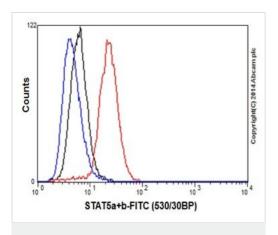


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling STAT5a + STAT5b using ab194898 at 1/2000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 was used as secondary. Counter stained with Hematoxylin. Inset image: negative control obtained using PBS instead of ab194898.

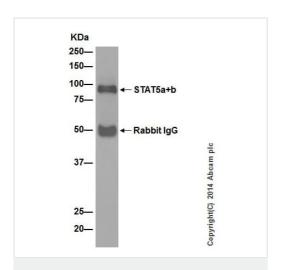
Nuclear and weak cytoplasmic staining on lymphocytes of rat spleen is observed.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

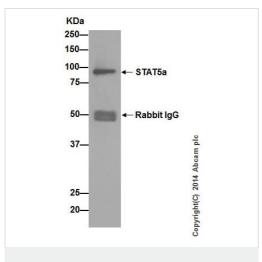
Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling STAT5a +STAT5b with ab194898 at 1/80 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

STAT5a and STAT5b were immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) cells with ab194898 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab194898 at 1/1000 dilution. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated, was used as secondary antibody at 1/1000 dilution.

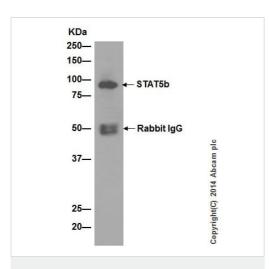
Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Immunoprecipitation - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

STAT5a + STAT5b were co-immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) cells with ab194898 at 1/100 dilution. Western blot was performed from the immunoprecipitate using <u>ab32043</u> (Rabbit monoclonal [E289] to STAT5a) at 1/1000 dilution. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated, was used as secondary antibody at 1/1000 dilution.

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



Immunoprecipitation - Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

STAT5a + STAT5b were co-immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) cells with ab194898 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab178941 (Rabbit monoclonal [EPR16671] to STAT5b) at 1/1000 dilution. Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated, was used as secondary antibody at 1/1000 dilution.

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



Anti-STAT5a + STAT5b antibody [EPR16671-40] (ab194898)

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