abcam

Product datasheet

Anti-STAT5b antibody [EPR16671] ab178941

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-STAT5b antibody [EPR16671]
Description	Rabbit monoclonal [EPR16671] to STAT5b
Host species	Rabbit
Specificity	This antibody shows no cross reactivity with STAT5a.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra), ChIP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: K562, HeLa, Jurkat, Daudi whole cell lysates. C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates. Mouse and Rat brain, heart, kidney and spleen lysates. Human fetal heart, kidney and spleen lysates. IHC-P: Rat colon, Mouse spleen and Human spleen tissues. ICC/IF: HeLa cells. IP: K562 whole cell extract. ChIP: T-47D 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	Liquid
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR16671
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab178941 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	★★★★★ **(4)**	1/5000. Detects a band of approximately 90 kDa (predicted molecular weight: 90 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/40.
Flow Cyt (Intra)		1/40.
ChIP		Use at an assay dependent concentration.

Target

Function	Carries out a dual function: signal transduction and activation of transcription. Mediates cellular responses to the cytokine KITLG/SCF and other growth factors. Binds to the GAS element and activates PRL-induced transcription.
Involvement in disease	Growth hormone insensitivity with immunodeficiency
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated in response to signaling via activated KIT, resulting in translocation to the nucleus. Tyrosine phosphorylated in response to signaling via activated FLT3; wild-type FLT3 results in much weaker phosphorylation than constitutively activated mutant FLT3. Alternatively, can be phosphorylated by JAK2. Phosphorylation at Tyr-699 by PTK6 or HCK leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates prolactin signaling pathway.
Cellular localization	Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

Images

Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

All lanes : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT5B knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

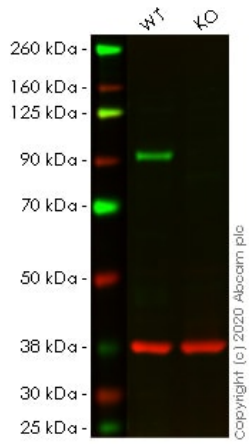
Secondary
All lanes : Goat anti-rabbit HRP at 0.2 µg/ml

Performed under reducing conditions.

Predicted band size: 90 kDa

ab178941 was shown to react with STAT5B in wild-type HeLa cells in Western blot with loss of signal observed in STAT5B knockout cell line **ab266006** (STAT5B knockout cell lysate **ab257710**). Wild-type HeLa and STAT5B knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab178941 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2µg/mL before imaging.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

All lanes : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/20000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT5B knockout HeLa cell lysate

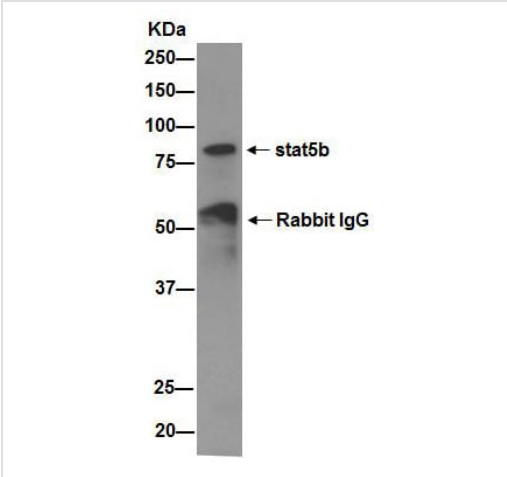
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

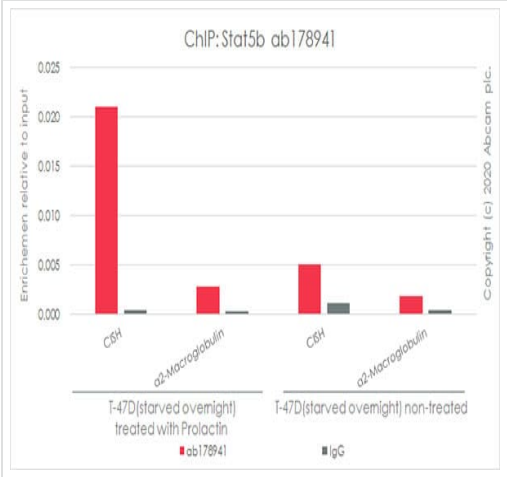
Lanes 1-2: Merged signal (red and green). Green - ab178941 observed at 90 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab178941 Anti-STAT5b antibody [EPR16671] was shown to specifically react with STAT5b in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab266006** (knockout cell lysate **ab257710**) was used. Wild-type and STAT5b knockout samples were subjected to SDS-PAGE. ab178941 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 20000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-STAT5b antibody [EPR16671] (ab178941)

Immunoprecipitation of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell extract using ab178941 at 1/40 dilution. Western blot detection of STAT5b utilised ab178941 at 1/2000 dilution and Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at 1/1000 dilution. The blocking and dilution buffer was 5% NFDM/TBST.

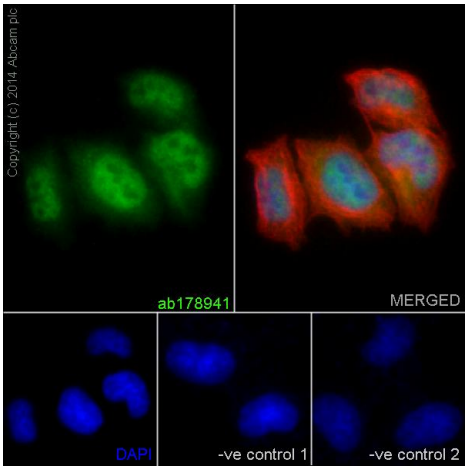


ChIP - Anti-STAT5b antibody [EPR16671] (ab178941)

Chromatin was prepared from T-47D (starved overnight) treated with Prolactin(10nM 30min) and T-47D(starved overnight) non-treated cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab178941 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µL of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers are from PMID: 15686596.

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

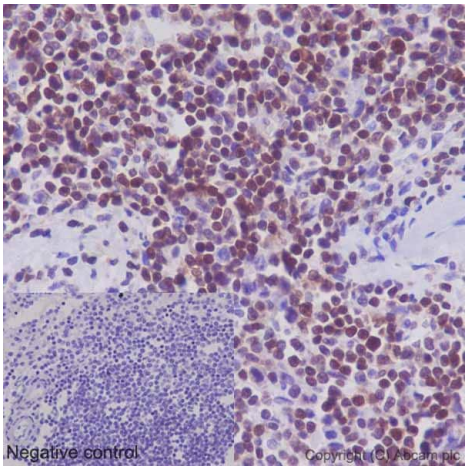


Immunocytochemistry/ Immunofluorescence - Anti-STAT5b antibody [EPR16671] (ab178941)

Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling STAT5b with ab178941 at 1/100 dilution. The cells were permeabilised with 0.1% Triton X-100. Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/200 dilution was used as the secondary antibody (green). Nuclear and cytoplasm staining is detected. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (Tubulin mouse mAb) at 1/500 and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/400 dilution (red).

The negative controls are as follows;

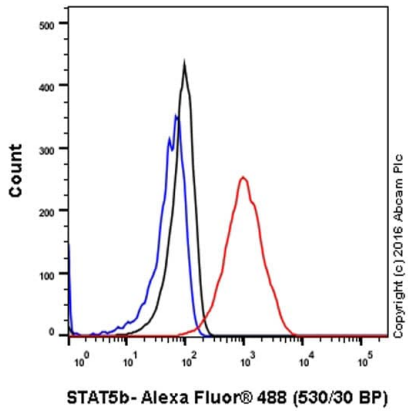
1. ab178941 at 1/100 dilution followed by Goat anti mouse IgG (Alexa Fluor®594) at 1/400 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by Goat anti rabbit IgG (Alexa Fluor®488) ar 1/200 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT5b antibody [EPR16671] (ab178941)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling STAT5b with ab178941 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus staining on lymphocytes of Human spleen is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

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

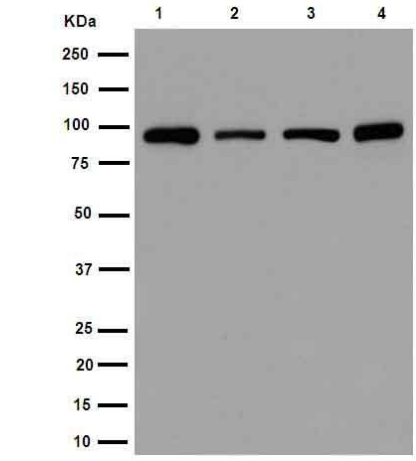


Flow Cytometry (Intracellular) - Anti-STAT5b antibody [EPR16671] (ab178941)

ab178941 staining STAT5bin the human cell line HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/40. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

All lanes : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/20000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysates
Lane 2 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates
Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates
Lane 4 : Daudi (Human Burkitt's lymphoma cell line) whole cell lysates

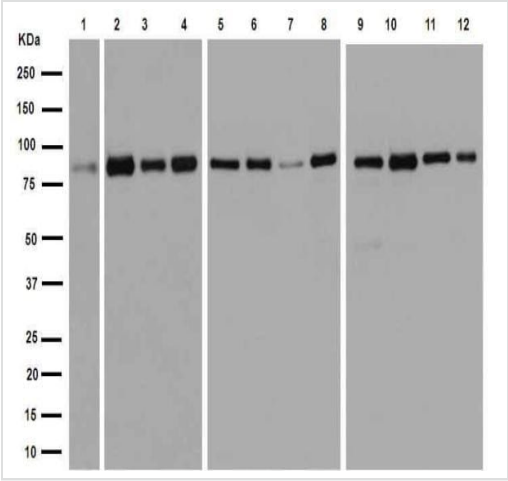
Lysates/proteins at 20 µg per lane.

Secondary

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

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

Blocking/dilution buffer: 5% NFDM/TBST.



Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

All lanes : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/5000 dilution

Lane 1 : Mouse brain lysates
Lane 2 : Mouse heart lysates
Lane 3 : Mouse kidney lysates
Lane 4 : Mouse spleen lysates
Lane 5 : Rat brain lysates
Lane 6 : Rat heart lysates
Lane 7 : Rat kidney lysates
Lane 8 : Rat spleen lysates
Lane 9 : C6 (Rat glial tumor cells) whole cell lysates
Lane 10 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysates
Lane 11 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates
Lane 12 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysates

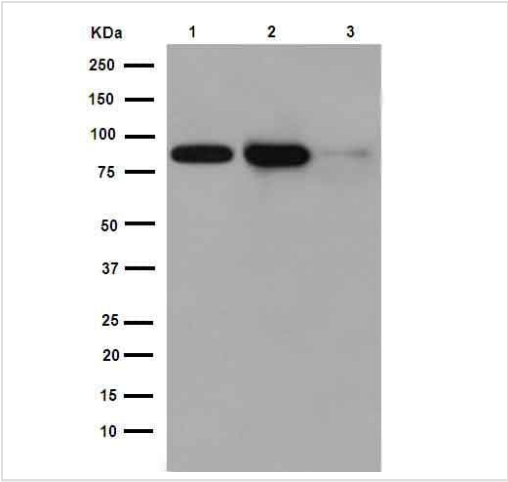
Lysates/proteins at 10 µg per lane.

Secondary

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

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

Blocking/dilution buffer: 5% NFDM/TBST.



Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

All lanes : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/20000 dilution

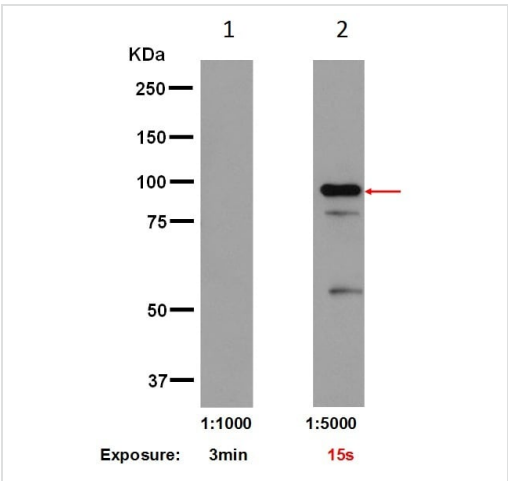
Lane 1 : Human fetal heart lysates
Lane 2 : Human fetal kidney lysates
Lane 3 : Human fetal spleen lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

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

Blocking/dilution buffer: 5% NFDM/TBST.



Western blot - Anti-STAT5b antibody [EPR16671] (ab178941)

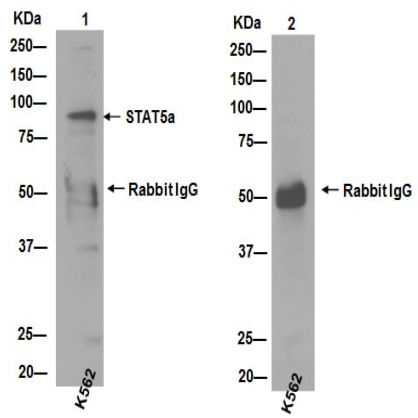
Lane 1 : Anti-STAT5b antibody [EPR16671] (ab178941) at 1/1000 dilution
Lane 2 : Anti-STAT5a antibody [E289] (**ab32043**) at 1/5000 dilution

All lanes : STAT5a recombinant protein

Developed using the ECL technique.

Predicted band size: 90 kDa

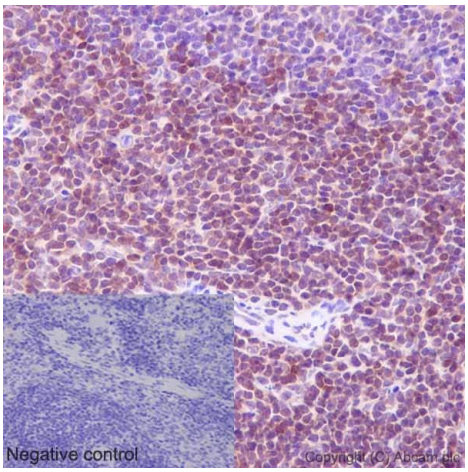
WB showing no cross reactivity with STAT5a.



Immunoprecipitation - Anti-STAT5b antibody [EPR16671] (ab178941)

Cross Immunoprecipitation of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell extract showing no cross reactivity with STAT5a. Protein captured by anti-STAT5a antibody (**ab32042**) was detected by the same antibody in WB (image 1) but not by anti-STAT5b, ab178941 (image 2).

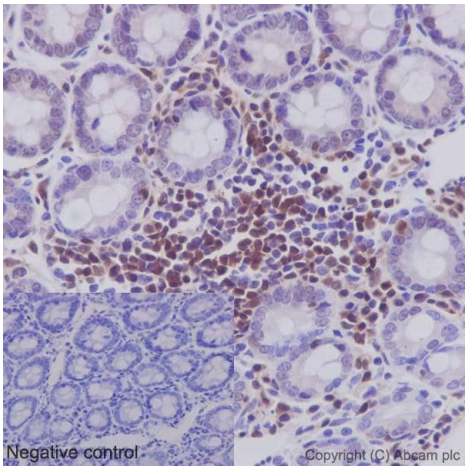
For WB detection, ab178941 was used at a 1/2000 dilution and Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at a 1/1000 dilution. The blocking and dilution buffer was 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT5b antibody [EPR16671] (ab178941)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling STAT5b with ab178941 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus staining on lymphocytes of Mouse spleen is detected. The negative control utilised PBS instead of primary antibody. Counter stained with Hematoxylin.

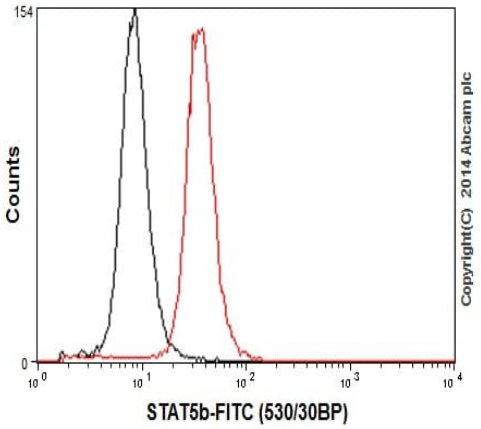
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT5b antibody [EPR16671] (ab178941)

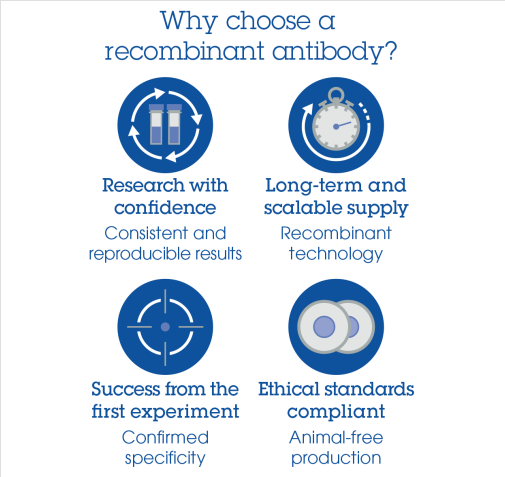
Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling STAT5b with ab178941 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus staining on lymphocytes and weak nucleus staining on gland epithelium of colon is detected. The negative control utilised PBS insead of primary antibody and the slide is counter stained with Hematoxylin.

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



Flow Cytometry (Intracellular) - Anti-STAT5b antibody [EPR16671] (ab178941)

Intracellular Flow Cytometry analysis of 2% paraformaldehyde K562 (Human chronic myelogenous leukemia cells from bone marrow) cellslabeling STAT5b with ab178941 at 1/60 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG (black line).



Anti-STAT5b antibody [EPR16671] (ab178941)

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