

Anti-STAT5b antibody [EPR16671] ab178941






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概述

产品名称	Anti-STAT5b抗体[EPR16671]
描述	兔单克隆抗体[EPR16671] to STAT5b
宿主	Rabbit
特异性	This antibody shows no cross reactivity with STAT5a.
经测试应用	适用于: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra), ChIP
种属反应性	与反应: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

性能

形式	Liquid
存放说明	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.
存储溶液	<p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>
纯度	Protein A purified
克隆	单克隆

克隆编号EPR16671

同种型IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab178941于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

功能	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.
疾病相关	Growth hormone insensitivity with immunodeficiency
序列相似性	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
翻译后修饰	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.
细胞定位	Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

图片



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

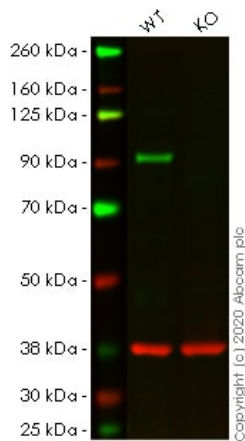
All lanes : Goat anti-rabbit HRP at 0.2 µg/ml

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.

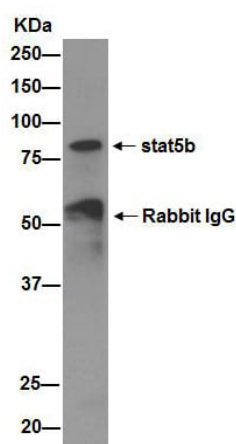
Performed under reducing conditions.

Predicted band size: 90 kDa

Observed band size: 90 kDa

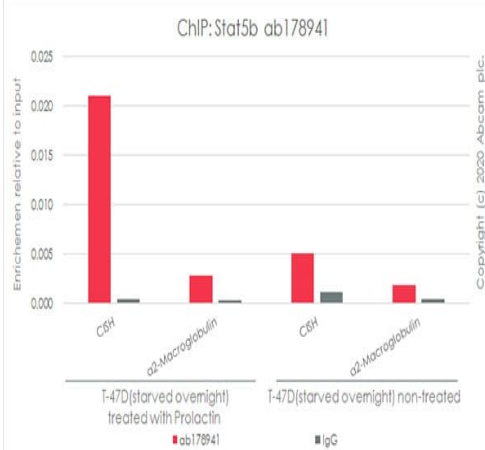
Lanes 1-2: Merged signal (red and green). Green - ab178941 observed at 90 kDa. Red - loading control **ab8245** observed at 37 kDa.

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)

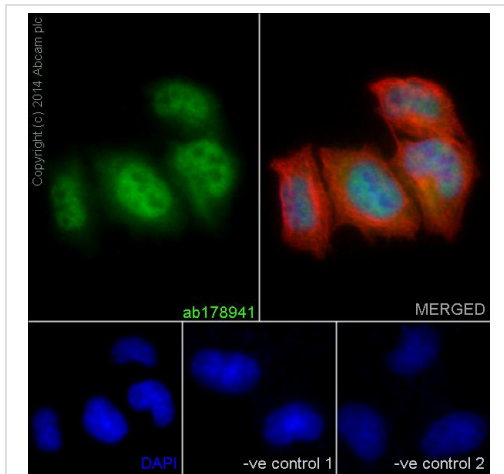
Chromatin was prepared from T-47D (starved overnight) treated with Prolactin(10nM 30min) and T-47D(starved overnight) non-treated cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

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

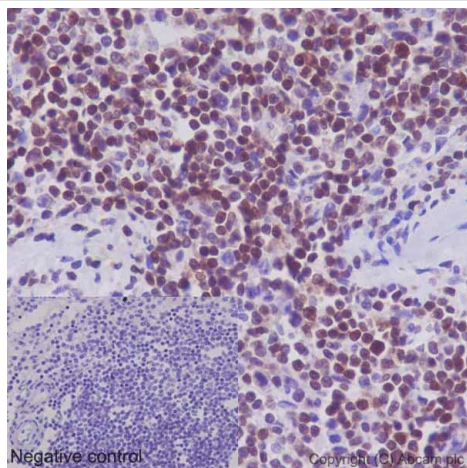


Immunocytochemistry/ Immunofluorescence - Anti-STAT5b antibody [EPR16671] (ab178941)

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;

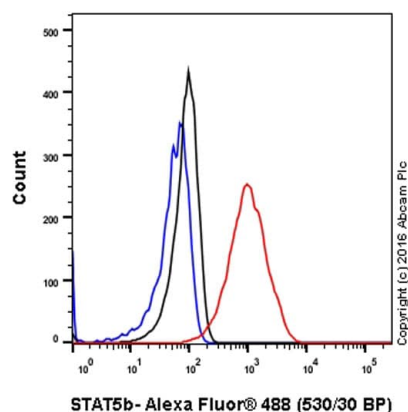
1. ab178941 at 1/100 dilution followed by Goat anti mouse IgG (Alexa Fluor®594) at 1/400 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by Goat anti rabbit IgG (Alexa Fluor®488) at 1/200 dilution.



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.

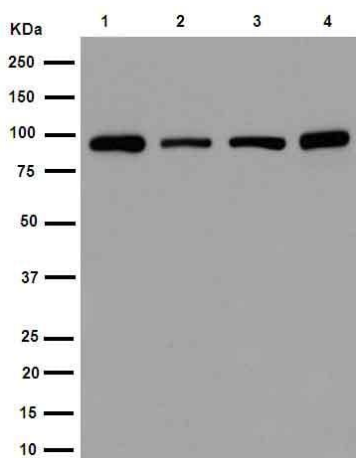


Flow Cytometry (Intracellular) - Anti-STAT5b antibody [EPR16671] (ab178941)

ab178941 staining STAT5b in 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.

Isotype 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.

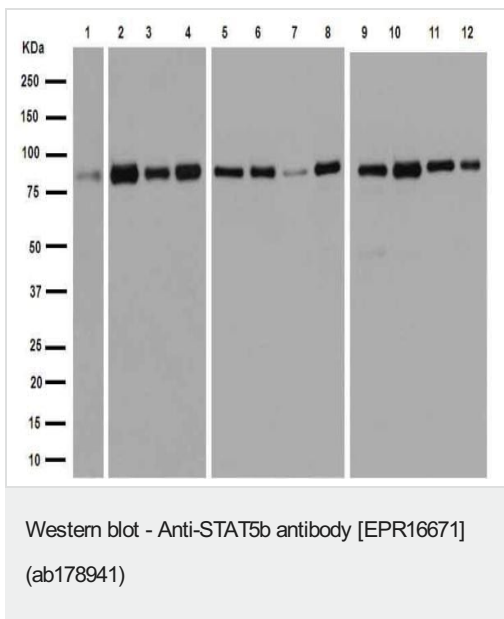
Secondary

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.



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 embryo fibroblast cells) whole cell lysates

Lysates/proteins at 10 µg per lane.

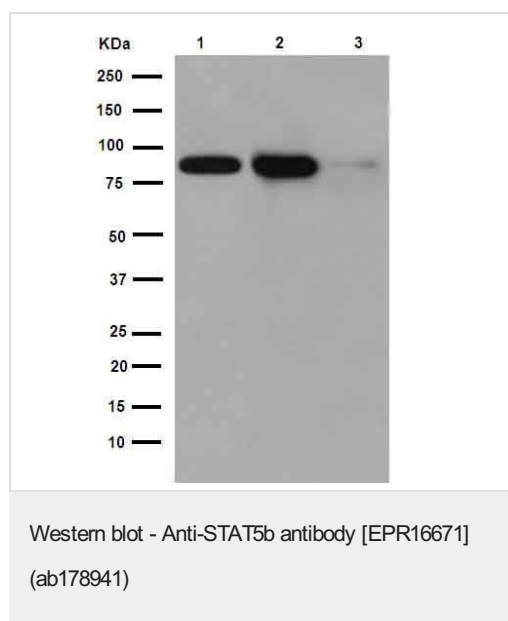
Secondary

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.



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.

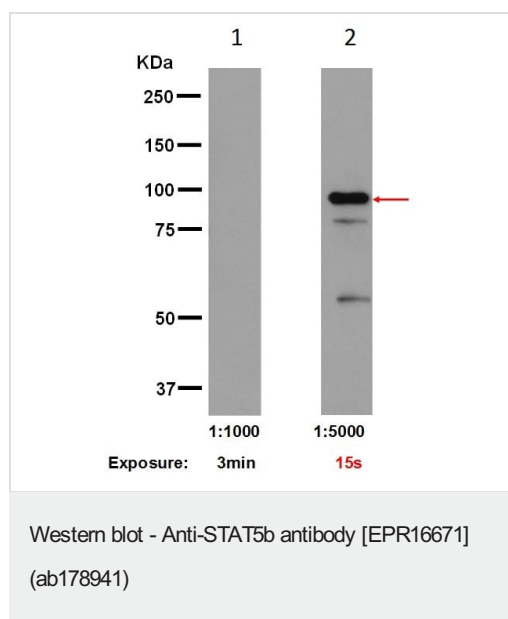
Secondary

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.



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

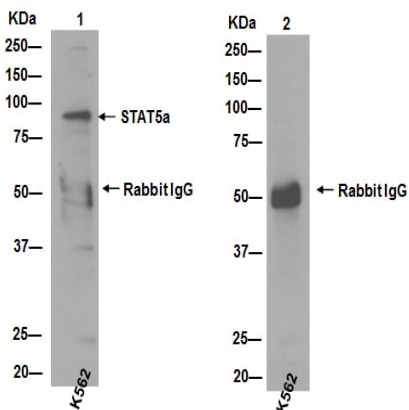
Lane 2 : Anti-STAT5a antibody [E289] ([ab32043](#)) at 1/5000 dilution

All lanes : STAT5a recombinant protein

Developed using the ECL technique.

Predicted band size: 90 kDa

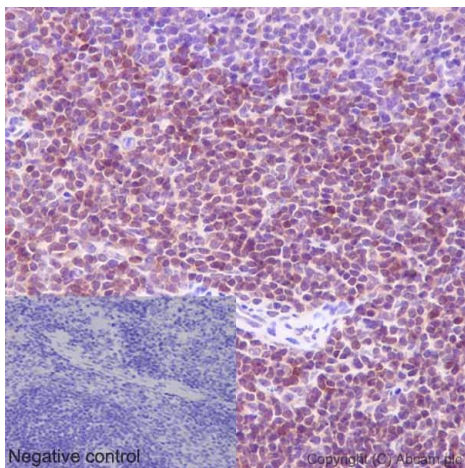
WB showing no cross reactivity with STAT5a.



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).

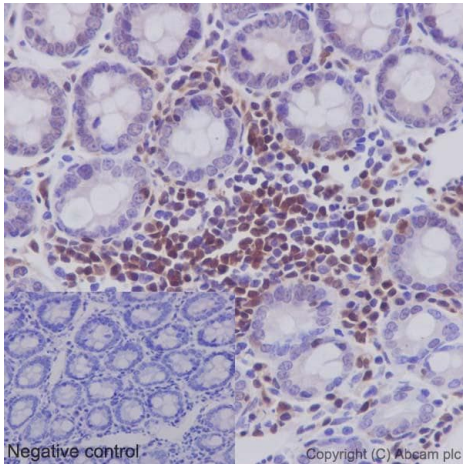
For WB detection, ab178941 was used at a 1/2000 dilution and Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at a 1/1000 dilution. The blocking and dilution buffer was 5% NFDM/TBST.



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.

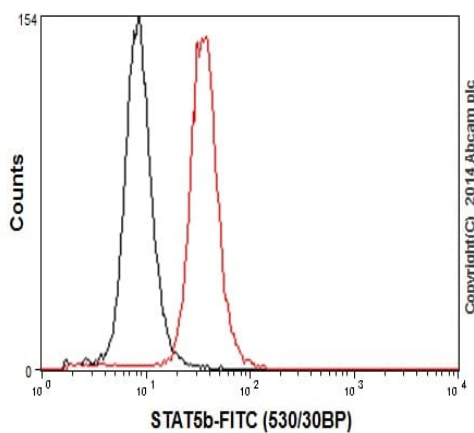
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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 instead 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) cells labeling 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).

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Anti-STAT5b antibody [EPR16671] (ab178941)

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