abcam

Product datasheet

# Anti-STAT3 antibody [EPR361] ab109085

敲除验证 重组 RabMAb

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## 概述

| | |
|---|---|
| **产品名称** | Anti-STAT3抗体[EPR361] |
| **描述** | 兔单克隆抗体[EPR361] to STAT3 |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** Flow Cyt (Intra), WB, IHC-P, ICC/IF<br>**不适用于:** IP |
| **种属反应性** | **与反应:** Mouse, Rat, Human |
| **免疫原** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | WB: HAP1, HEK293, A431, Raji, HeLa, HaCaT, NIH/3T3, C2C12, and SH-SY5Y cell lysates. Human, mouse, and rat brain, mouse heart, and rat kidney tissue lysates. ICC/IF: HeLa and A459 cells. IHC-P: Human pancreas tissue. Flow Cyt (intra): HeLa cells. |
| **常规说明** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 性能

| | |
|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C. |
| **存储溶液** | pH: 7.20<br>Preservative: 0.01% Sodium azide<br>Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | EPR361 |

**同种型** IgG

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab109085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 |
|---|---|---|
| **Flow Cyt (Intra)** | | 1/300. |
| **WB** | | 1/1000 - 1/10000. Predicted molecular weight: 88 kDa. |
| **IHC-P** | | 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.<br>See **IHC antigen retrieval protocols**. |
| **ICC/IF** | | 1/150. |

**应用说明** Is unsuitable for IP.

## 靶标

**功能** Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transctivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.

**组织特异性** Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.

**疾病相关** Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant
Autoimmune disease, multisystem, infantile-onset

**序列相似性** Belongs to the transcription factor STAT family.
Contains 1 SH2 domain.

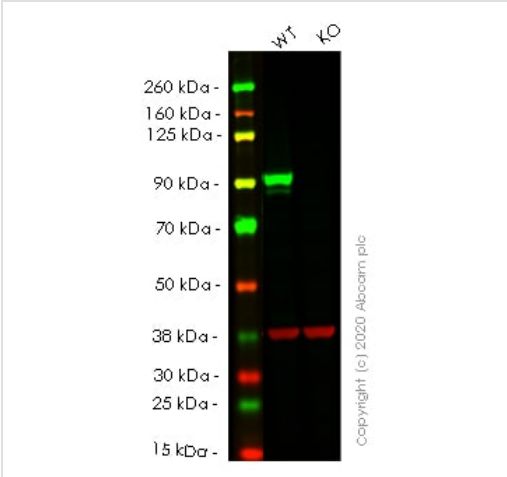
**翻译后修饰** Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF, KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation is important for the formation of stable DNA-binding STAT3 homodimers and maximal transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by

PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

**细胞定位**

Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

## 图片




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** STAT3 knockout HeLa cell lysate

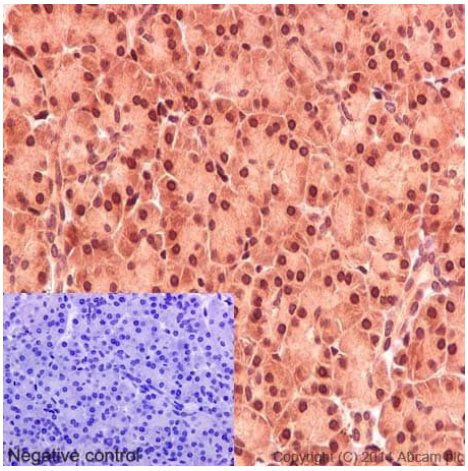
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 88 kDa
**Observed band size:** 92 kDa

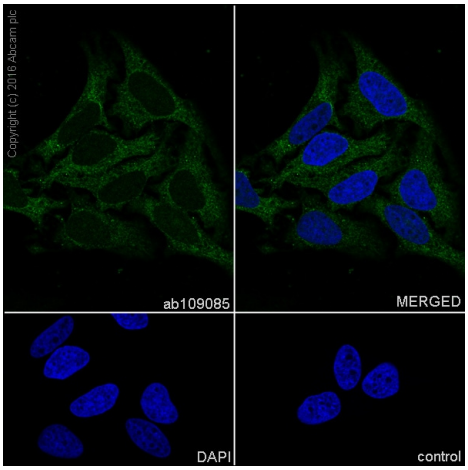
**Lanes 1- 2:** Merged signal (red and green). Green - ab109085 observed at 92 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109085 was shown to react with STAT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255436** (knockout cell lysate **ab263797**) was used. Wild-type HeLa and STAT3 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109085 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



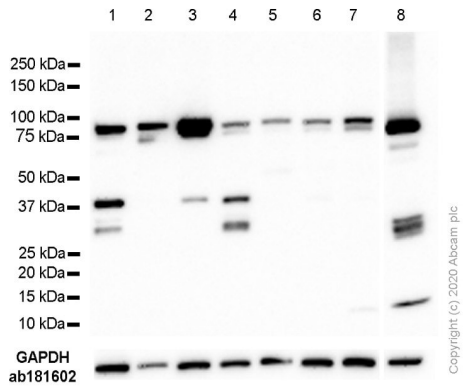

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 antibody [EPR361] (ab109085)

Immunohistochemical staining of paraffin embedded human pancreas with purified ab109085 at a working dilution of 1 in 100. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.




Immunocytochemistry/ Immunofluorescence - Anti-STAT3 antibody [EPR361] (ab109085)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling STAT3 with Purified ab109085 at 1/250 dilution (5 µg/ml). Cells were fixed with 4% PFA and permeabilized with 0.1% tritonX-100. **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) at 1/1000 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/1000 dilution

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
**Lane 2 :** HaCaT (Human skin keratinocyte) whole cell lysate
**Lane 3 :** NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
**Lane 4 :** C2C12 (Mouse myoblasts cell line) whole cell lysate
**Lane 5 :** Human brain lysate
**Lane 6 :** Mouse brain lysate
**Lane 7 :** Rat brain lysate
**Lane 8 :** Rat liver lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

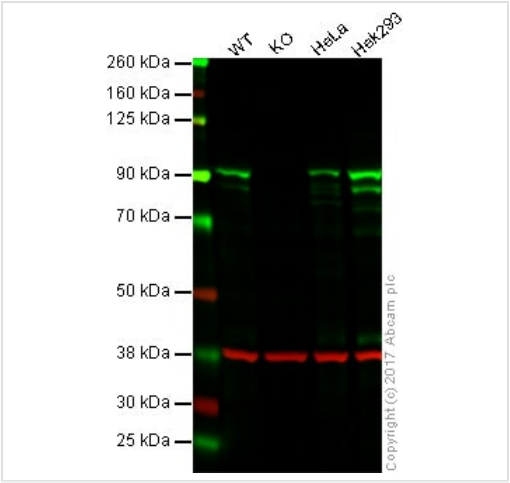
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 88 kDa
**Observed band size:** 92 kDa
**Additional bands at:** 32 kDa, 40 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 50 seconds

**Blocking/Diluting buffer:** 5% NFDM/TBST.

Rabbit monoclonal [EPR16891] to GAPDH (**ab181602**) used as loading control.




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/1000 dilution

**Lane 1 :** Wild-type HAP1 whole cell lysate
**Lane 2 :** STAT3 knockout HAP1 whole cell lysate
**Lane 3 :** HeLa whole cell lysate
**Lane 4 :** HEK293 whole cell lysate

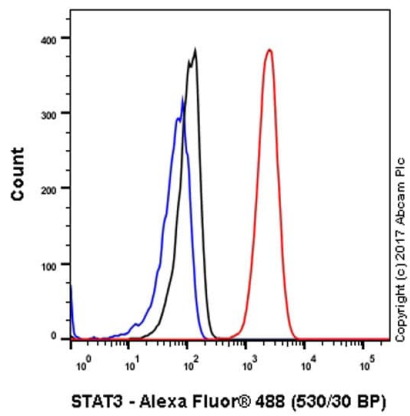
Lysates/proteins at 20 µg per lane.

**Predicted band size:** 88 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab109085 observed at 92 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

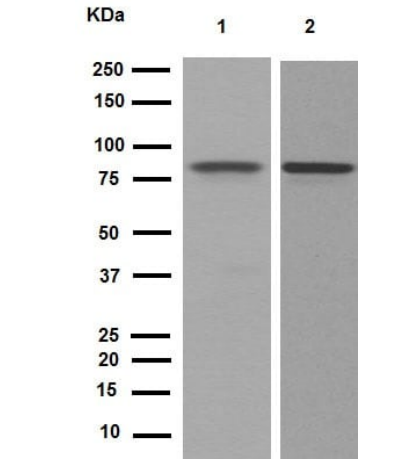
Ab109085 was shown to specifically react with STAT3 in wild-type cells as signal was lost in STAT3 knockout HAP1 cells. Wild-type and STAT3 knockout samples were subjected to SDS-PAGE. Ab109085 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary

antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.




Flow Cytometry (Intracellular) - Anti-STAT3 antibody [EPR361] (ab109085)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling STAT3 (red) with ab109085 at a 1/300 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluorr® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/2000 dilution (purified)

**Lane 1 :** Mouse heart tissue lysate
**Lane 2 :** Rat brain tissue lysate

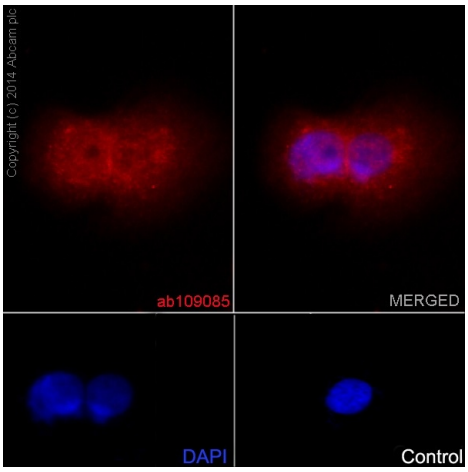
Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 88 kDa
**Observed band size:** 88 kDa

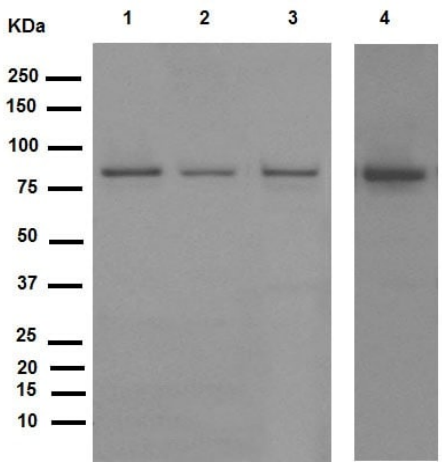
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST




Immunocytochemistry/ Immunofluorescence - Anti-STAT3 antibody [EPR361] (ab109085)

Immunofluorescence staining of A549 cells with purified ab109085 at a working dilution of 1 in 150, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 555 goat anti rabbit (**ab150078**), used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, purified ab109085 was used at a dilution of 1/200 followed by an Alexa Fluor® 488 goat anti-mouse antibody at a dilution of 1/500.




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/2000 dilution (purified)

**Lane 1 :** A431 cell lysate
**Lane 2 :** Raji cell lysate
**Lane 3 :** HeLa cell lysate
**Lane 4 :** SH-S5SY (Human neuroblastoma cell line from bone marrow) cell lysate

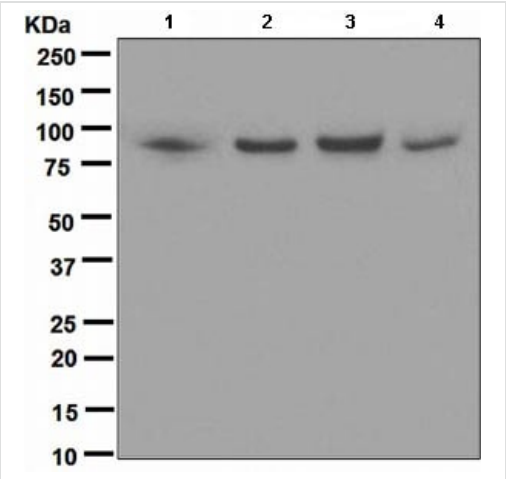
Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** HRP goat anti-rabbit (H+L) at 1000 mg/ml

**Predicted band size:** 88 kDa
**Observed band size:** 88 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST




Western blot - Anti-STAT3 antibody [EPR361] (ab109085)

**All lanes :** Anti-STAT3 antibody [EPR361] (ab109085) at 1/1000 dilution (unpurified)

**Lane 1 :** A431 cell lysate
**Lane 2 :** Raji cell lysate
**Lane 3 :** HeLa cell lysate
**Lane 4 :** SH-SY5Y cell lysate

Lysates/proteins at 10 µg per lane.

**Predicted band size:** 88 kDa


Anti-STAT3 antibody [EPR361] (ab109085)



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