abcam

Product datasheet

# Anti-STAT3 (phospho Y705) antibody [EPR23968-52] ab267373

敲除验证 重组 RabMAb

**24 References** 10 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-STAT3 (phospho Y705)**抗体**[EPR23968-52] |
| **描述** | **兔单克隆抗体**[EPR23968-52] to STAT3 (phospho Y705) |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** WB, IHC-P, IP, Flow Cyt (Intra), ChIP, Dot blot, ICC/IF |
| **种属反应性** | **与反应:** Human |
| **免疫原** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | WB: HeLa serum starved overnight, treated 30 mins 50ng/ml IFN alpha; HepG2 serum starved overnight, treated 30 mins, 100ng/ml IL-6; Jurkat treated 30 mins, 50ng/ml IFN alpha. IHC-P: Human endometrial carcinoma, tonsil tissue. ICC/IF: HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min. Flow Cyt (intra): Jurkat treated (30 mins) with 50ng/ml IFN alpha. IP: Jurkat treated (30 mins) with 50ng/ml IFN alpha. Dot: STAT3 phospho Y705 peptide (aa700-710) ChIP: HepG2 (starved overnight) treated with IL-6. |
| **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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **存储溶液** | Preservative: 0.01% Sodium azide<br>Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |

| | |
|---|---|
| **克隆编号** | EPR23968-52 |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab267373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** | | 1/1000. Predicted molecular weight: 88 kDa. |
| **IHC-P** | | 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **IP** | | 1/30. |
| **Flow Cyt (Intra)** | | 1/500. |
| **ChIP** | | Use 5 µg for 25 µg of chromatin. |
| **Dot blot** | | 1/1000. |
| **ICC/IF** | | 1/500. |

## 靶标

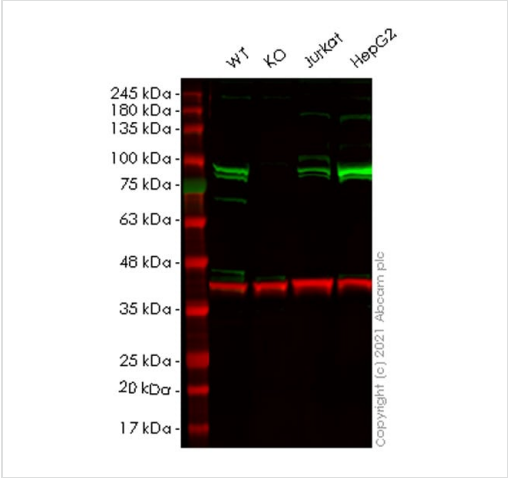
| | |
|---|---|
| **功能** | Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular 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<br>Autoimmune disease, multisystem, infantile-onset |
| **序列相似性** | Belongs to the transcription factor STAT family.<br>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 (phospho Y705) antibody [EPR23968-52] (ab267373)

**All lanes :** Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa (human cervix adenocarcinoma epithelial cell) serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate
**Lane 2 :** STAT3 knockout HeLa serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate
**Lane 3 :** Jurkat (human t cell leukemia cell line from peripheral blood) treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate
**Lane 4 :** HepG2 (human hepatocellar carcinoma epithelial cell) serum starved overnight, then treated with 100 ng/ml IL-6 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

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

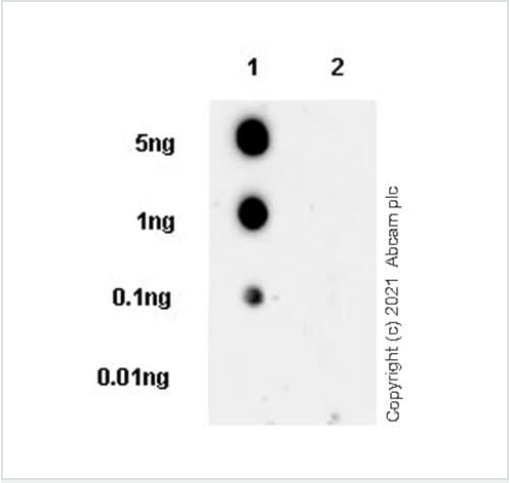
Blocking and diluting buffer and concentration: Intercept® (TBS)

Blocking Buffer diluted with an equal volume of 0.1% TBS.

**Lanes 1-4:** Merged signal (red and green). Green - ab267373 observed at 88 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

**Lanes 1-2:** ab267373 Anti-STAT3 (phospho Y705) antibody [EPR23968-52] was shown to specifically react with STAT3 in wild-type serum starved and then IFN alpha treated HeLa cells. Loss of signal was observed when serum starved and then IFN alpha treated knockout cell line **ab255436** (knockout cell lysate **ab263797**) was used. Wild-type and STAT3 knockout samples were subjected to SDS-PAGE. ab267373 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight at 1 in 1000 dilution 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 10000 dilution for 1 hour at room temperature before imaging.

Lysates loaded onto lanes 3-4 were made freshly and used in WB immediately to minimize protein degradation.




Dot Blot - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

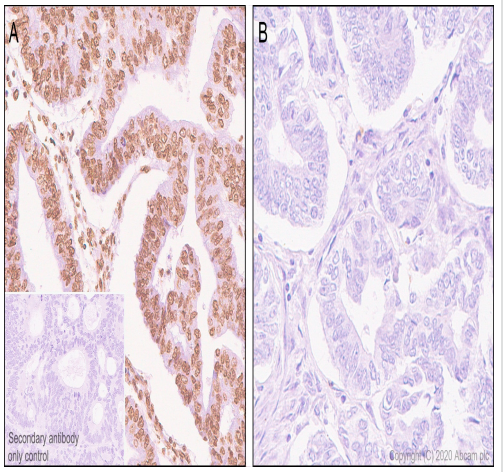
Dot blot analysis of STAT3 (phospho Y705) using ab267373 at 1/1000 followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1:100,000 dilution.

**Lane 1**: STAT3 phospho Y705 peptide (aa700-710)

**Lane 2**: Unmodified STAT3 peptide (aa698-710)

Exposure time: 3 minutes.

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

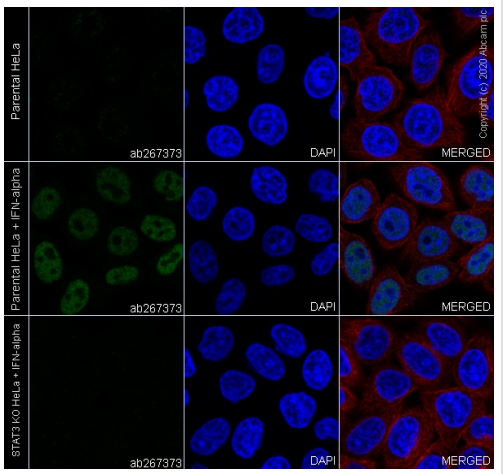



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue labeling STAT3 (phospho Y705) with ab267373 at 1/500 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human endometrial carcinoma without alkaline phosphatase treatment (image A) is observed. No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab267373 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

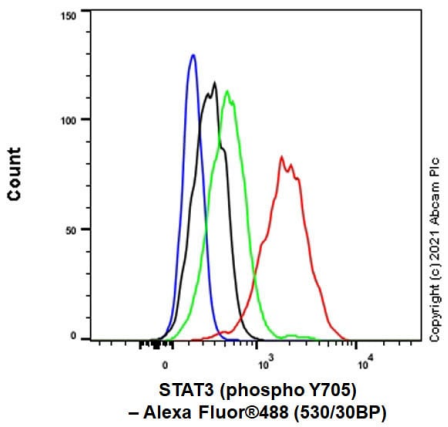



Immunocytochemistry/ Immunofluorescence - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 100% Methanol-permeabilized STAT3 KO HeLa cells labelling STAT3 (phospho Y705) with ab267373 at 1/500 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green).

Confocal image showing increased nuclear staining in parental HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min, and no staining in STAT3 knockout HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min.

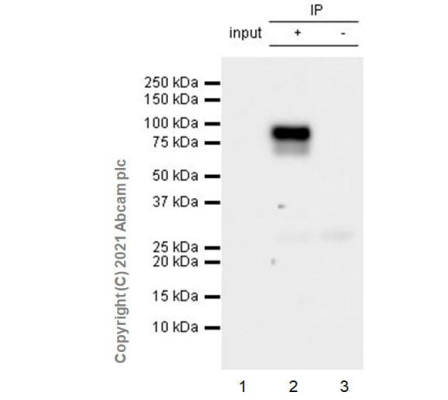
**ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).




Flow Cytometry (Intracellular) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Jurkat (Human T cell leukemia T lymphocyte) treated with 50 ng/ml IFN-alpha for 30min (Red)/ Untreated control (Green) cells labelling STAT3 (phospho Y705) with ab267373 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077** at 1/2000 dilution was used as the secondary antibody.




Immunoprecipitation - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

STAT3 (phospho Y705) was immunoprecipitated from 0.35 mg Jurkat (human t cell leukemia cell line from peripheral blood) treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate 10 µg with ab267373 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267373 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

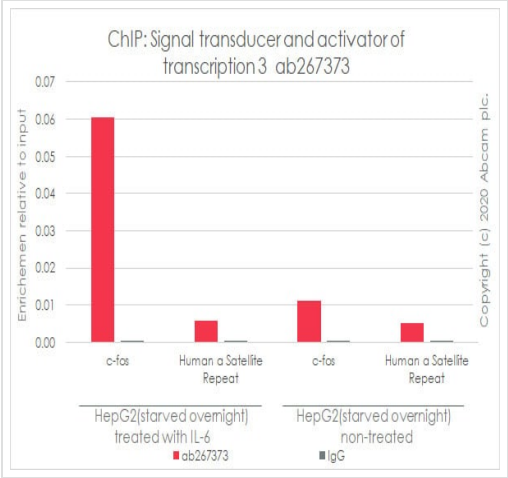
**Lane 1**: Jurkat treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate 10 µg

**Lane 2**: ab267373 IP in Jurkat treated with 50 ng/ml IFN alpha for 30 minutes whole cell lysate

**Lane 3**: Rabbit monoclonal IgG (**ab172730**) instead of ab267373 in Jurkat treated with 50 ng/ml IFN alpha for 30 minutes whole cell lysate

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

Exposure time: 15 seconds.

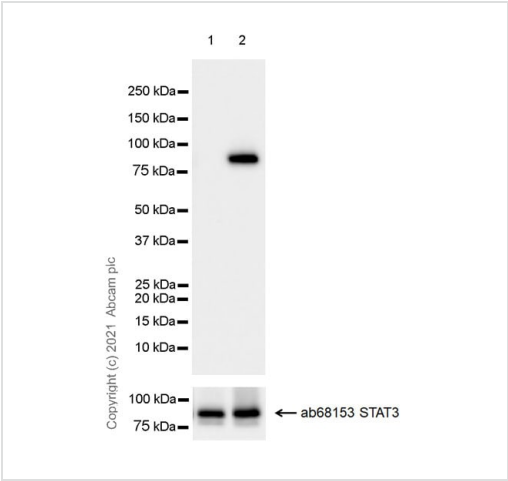



ChIP - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Chromatin was prepared from HepG2 (starved overnight) treated with IL-6 cells and HepG2 (starved overnight) non-treated 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 ab267373 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
Primers are purchased from competitor.
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol




Western blot - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

**All lanes :** Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

**Lane 1 :** HepG2 (human hepatocellular carcinoma epithelial cell) serum starved overnight, whole cell lysate
**Lane 2 :** HepG2 serum starved overnight, then treated with 100 ng/ml IL-6 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

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

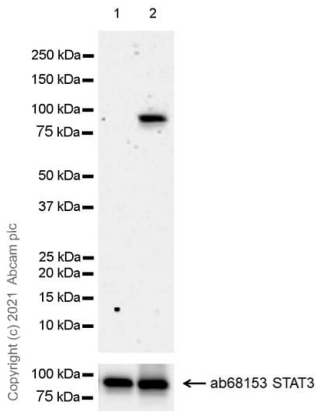
**Predicted band size:** 88 kDa
**Observed band size:** 88 kDa

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

IL-6 treatment induces phosphorylation of STAT3 at Tyr705 (PMID: 28676732).

Lysates were made freshly and used in WB immediately to minimize protein degradation.

Exposure time: 26 seconds.




Western blot - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

**All lanes :** Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

**Lane 1 :** HeLa (human cervix adenocarcinoma epithelial cell) serum starved overnight, whole cell lysate
**Lane 2 :** HeLa serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

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

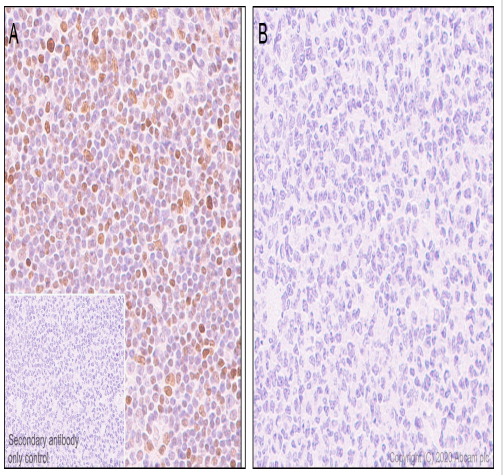
**Predicted band size:** 88 kDa
**Observed band size:** 88 kDa

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

IFN alpha treatment induces phosphorylation of STAT3 at Tyr705 (PMID: 21957129).

Lysates were made freshly and used in WB immediately to minimize protein degradation.

Exposure time: 3 minutes.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling STAT3 (phospho Y705) with ab267373 at 1/500 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human tonsil without alkaline phosphatase treatment (image A) is observed. No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab267373 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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