# abcam

## Product datasheet

# Anti-STAT6 (phospho Y641) antibody [EPR22599-78] ab263947

Recombinant RabMAb

14 References 12 Images

Overview

Product name Anti-STAT6 (phospho Y641) antibody [EPR22599-78]

**Description** Rabbit monoclonal [EPR22599-78] to STAT6 (phospho Y641)

Host species Rabbit

**Specificity** STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and

IL-13.

**Tested applications** Suitable for: IP, Dot blot, WB, IHC-P, Flow Cyt (Intra)

Unsuitable for: ChIP or ICC/IF

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**Positive control** IHC-P: Human kidney tissue, Rat liver tissue and Mouse kidney tissue; WB: Daudi serum IL-4,

2.4G2 and RAW 264.7 serums starved for 24 hours, then treated with 100 ng/ml;IP: RAW 264.7

and Daudi lysates; Flow Cyt (intra): RAW 264.7 and Daudi 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 specificityLong-term security of supply

- Animal-free production

For more information see here.

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **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 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

1

Purity Protein A purified

**Clonality** Monoclonal

Clone number EPR22599-78

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab263947 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
IP		1/30.
Dot blot		1/1000.
WB		1/1000. Predicted molecular weight: 94 kDa.
IHC-P		1/5000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/500.

**Application notes** Is unsuitable for ChIP or ICC/IF.

**Target** 

**Function** Carries out a dual function: signal transduction and activation of transcription. Involved in

interleukin-4 signalling.

**Sequence similarities**Belongs to the transcription factor STAT family.

Contains 1 SH2 domain.

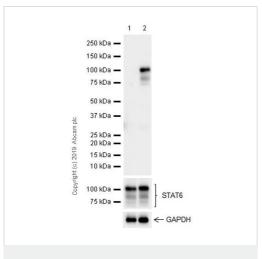
Post-translational

modifications

Tyrosine phosphorylated following stimulation by IL-4 and IL-3.

**Cellular localization** Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

#### **Images**



Western blot - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

**All lanes :** Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947) at 1/1000 dilution

**Lane 1 :** RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) serum starved for 24 hours, whole cell lysate at 10  $\mu g$ 

Lane 2: RAW264.7 serum starved for 24 hours, then treated with 100 ng/ml mlL-4 for 15 minutes, whole cell lysate 10 ug

#### Secondary

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

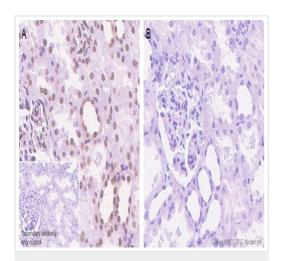
**Predicted band size:** 94 kDa **Observed band size:** 110,94 kDa

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

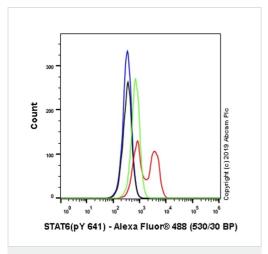
Phosphorylation of STAT6 at Y641 can be induced by IL-4 treatment (PMID: 21411736,15044251) The molecular weight observed is consistent with what has been described in the literature (PMID: 24708771).

STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.

Exposure time: 15 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)



Flow Cytometry (Intracellular) - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

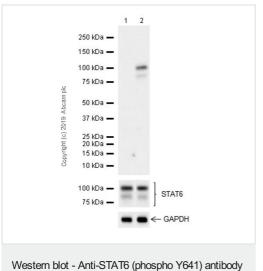
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling STAT6 (phospho Y641) with ab263947 at 1:5000 dilution (0.106 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human kidney (panel A), no staining after alkaline phosphatase treatment (panel B. PMID: 8085155, 16181056). The section was incubated with ab263947 for 15 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Heat mediated antigen retrieval with citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min (Red) / Untreated control (Green) cells labelling STAT6 (phospho Y641) with ab263947 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Black)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.

STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.



Western blot - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947) **All lanes :** Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947) at 1/5000 dilution

Lane 1: 2.4G2 (rat B cell lymphoma B lymphocyte) serum starved for 24 hours, whole cell lysate at 10 µg

**Lane 2**: 2.4G2 serum starved for 24 hours, then treated with 100 ng/ml rlL-4 for 15 minutes, whole cell lysate 10 ug

#### **Secondary**

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

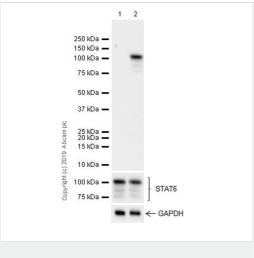
**Predicted band size:** 94 kDa **Observed band size:** 110,94 kDa

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

Phosphorylation of STAT6 at Y641 can be induced by IL-4 treatment (PMID: 21411736,15044251) The molecular weight observed is consistent with what has been described in the literature (PMID: 24708771).

STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.

Exposure time: 37 seconds



Western blot - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

**All lanes**: Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947) at 1/5000 dilution

Lane 1 : Daudi (human Burkitts lymphoma lymphoblast) serum starved for 24 hours, whole cell lysate at 10 µg

Lane 2: Daudi serum starved for 24 hours, then treated with 100 ng/ml IL-4 for 15 minutes, whole cell lysate 10 ug

#### Secondary

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

Predicted band size: 94 kDa

Observed band size: 110,75,81 kDa

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

Phosphorylation of STAT6 at Y641 can be induced by IL-4 treatment (PMID: 21411736,15044251) This antibody recognizes three isoforms of STAT6, isoform 1 (110 kDa), isoform 2 (75 kDa), isoform 3 (81 kDa), which is consistent with the Uniprot annotation. STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.

Exposure time: 15 seconds

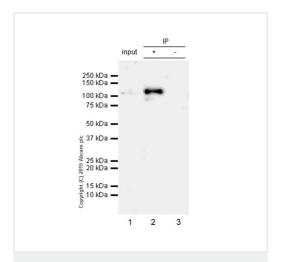
1 2
5ng
1ng
0.1ng
0.01ng

Dot Blot - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

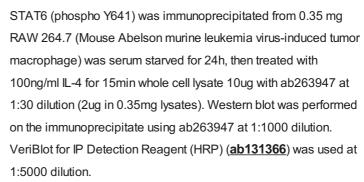
Dot blot analysis of STAT6 (phospho Y641) peptide (Lane 1 STAT6 non-phospho peptide peptide (Lane 2), labelling STAT 6 (pY641) with purified ab263947 at a dilution of 1/1000. <a href="mailto:ab97051"><u>ab97051</u></a> (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 26 seconds.



Immunoprecipitation - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)



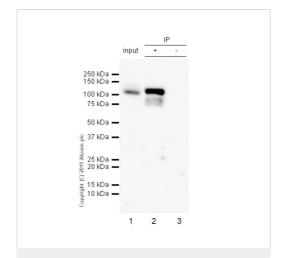
Lane 1: RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate 10ug

Lane 2: ab263947 IP in RAW 264.7 was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate

Lane 3: Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of ab263947 in RAW 264.7 was serum starved for 24h, then treated with 100ng/ml  $\mathbb{L}$ -4 for 15min whole cell lysate

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

Exposure time: 2 min



Immunoprecipitation - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

STAT6 (phospho Y641) was immunoprecipitated from 0.35 mg Daudi (Human Burkitt's lymphoma lymphoblast) was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate 10ug with ab263947 at 1:30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab263947 at 1:1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1:5000 dilution.

Lane 1: Daudi (Human Burkitt's lymphoma lymphoblast) was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate 10ug

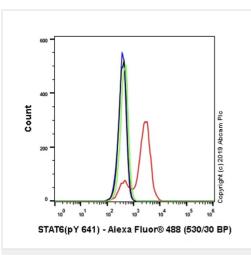
Lane 2: ab263947 IP in Daudi was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate

Lane 3: Rabbit monoclonal IgG ( $\underline{ab172730}$ ) instead of ab263947 in Daudi was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min whole cell lysate

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

STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.

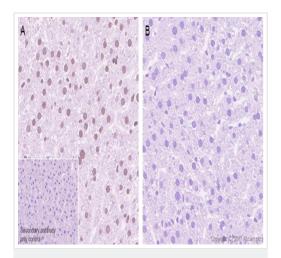
Exposure time: 1 min



Flow Cytometry (Intracellular) - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Daudi (Human Burkitt's lymphoma lymphoblast) was serum starved for 24h, then treated with 100ng/ml IL-4 for 15min (Red)/Untreated control (Green) cells labelling STAT6 (phospho Y641) with ab263947 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730)isotype control (black)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.

STAT6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13.

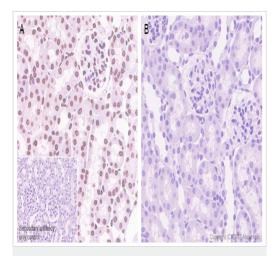


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

Immunohistochemical analysis of paraffin-embedded Rat liver tissue labeling STAT6 (phospho Y641) with ab263947 at 1:5000 dilution (0.106 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on rat liver (panel A), no staining after alkaline phosphatase treatment (panel B). The section was incubated with ab263947 for 15 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Heat mediated antigen retrieval with citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

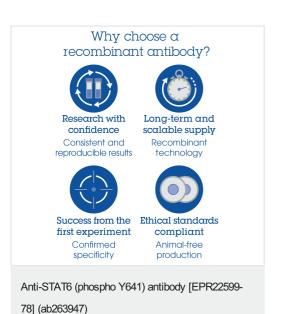


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 (phospho Y641) antibody [EPR22599-78] (ab263947)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling STAT6 (phospho Y641) with ab263947 at 1/5000 dilution (0.106 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse kidney (panel A), no staining after alkaline phosphatase treatment (panel B, PMID: 23155424). The section was incubated with ab263947 for 15 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Heat mediated antigen retrieval with citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



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