abcam

Product datasheet

Anti-STAT6 antibody [YE361] ab32520

Recombinant RabMAb

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Overview

Product name Anti-STAT6 antibody [YE361]

Description Rabbit monoclonal [YE361] to STAT6

Host species Rahhit

Specificity This antibody does not cross-react with other Stat family members.

Tested applications Suitable for: ChIC/CUT&RUN-seq, Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide within Human STAT6 aa 800 to the C-terminus (C terminal). The exact

> sequence is proprietary. Database link: P42226

Positive control WB: Raji, NIH/3T3 and RAW 264.7 cell lysates. IHC-P: Human skin carcinoma, kidney, transitional

> cell carcinoma of bladder, glioma and solitary fibrous tumor tissues; Mouse kidney tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: NIH/3T3 cell lysate. ChIC/CUT&RUN-Seq: HeLa 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

Properties

Form

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number YE361

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab32520 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
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	**** <u>(1)</u>	1/1000 - 1/2000. Detects a band of approximately 100 kDa (predicted molecular weight: 94 kDa).
IHC-P		1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		1/100.
IP		1/20 - 1/80.

Target

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

interleukin-4 signalling.

Sequence similaritiesBelongs to the transcription factor STAT family.

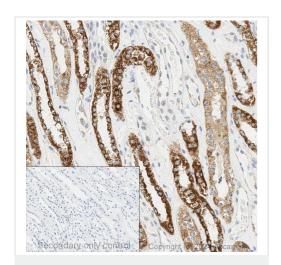
Contains 1 SH2 domain.

Post-translational modifications

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

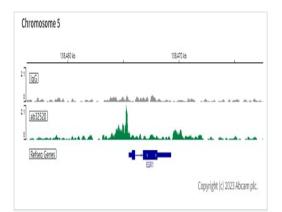
Cellular localization Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemical analysis of formalin fixed paraffin embedded human kidney labelling STAT6 with ab32520 at a concentration of 0.79µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 min with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5. ab32520 was incubated at 37°C for 16 min. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

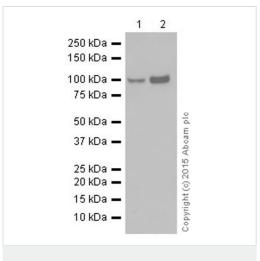


ChIC/CUT&RUN sequencing - Anti-STAT6 antibody [YE361] (ab32520)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5×10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and $5\mu g$ of ab32520 [YE361]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> is also shown.

Additional screenshots of mapped reads can be downloaded here.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-STAT6 antibody [YE361] (ab32520)

All lanes : Anti-STAT6 antibody [YE361] (ab32520) at 1/1000 dilution (purified)

Lane 1: NIH/3T3 (mouse embyro fibroblast cell line) cell lysate

Lane 2: RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate

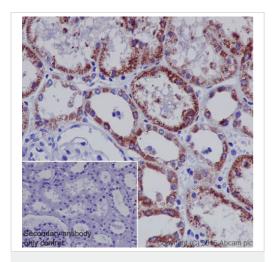
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit lgG (H+L) at 1/20000 dilution

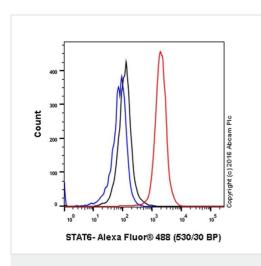
Predicted band size: 94 kDa **Observed band size:** 100 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



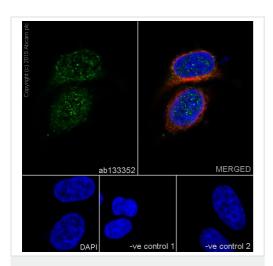
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemical staining of paraffin embedded human kidney with purified ab32520 at a working dilution of 1/50. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed 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.



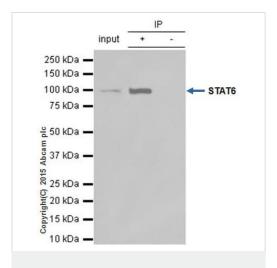
Flow Cytometry (Intracellular) - Anti-STAT6 antibody [YE361] (ab32520)

Intracellular Flow Cytometry analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling STAT6 with purified ab32520 at 1/30 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



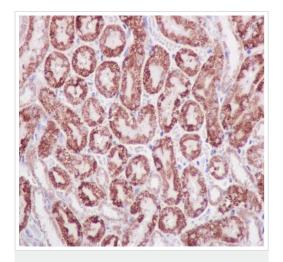
Immunocytochemistry/ Immunofluorescence - Anti-STAT6 antibody [YE361] (ab32520)

Immunofluorescence staining of HeLa (human epithelial cell line from cervix adenocarcinoma) cells with purified ab32520 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor[®] 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32520 was used at a dilution of 1/500 followed by an Alexa Fluor[®] 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor[®] 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



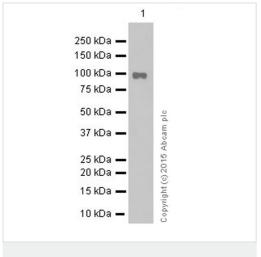
ab32520 (purified) at 1/20 immunoprecipitating STAT6 in 10 μ g NIH/3T3 (mouse embyro fibroblast cell line; Lanes 1 and 2, observed at 100 kDa). Lane 3 - PBS. For western blotting, HRP Veriblot for IP (ab131366) was used for detection (1/10 000). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

Immunoprecipitation - Anti-STAT6 antibody [YE361] (ab32520)

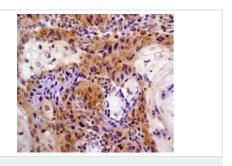


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labelling STAT6 with unpurified ab32520 at a dilution of 1/1000. HRP goat antirabbit (ab97051) was used at a dilution of 1/500. The antigen retrieval solution was Tris-EDTA buffer, pH 9.0.



Western blot - Anti-STAT6 antibody [YE361] (ab32520)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Anti-STAT6 antibody [YE361] (ab32520) at 1/20000 dilution (purified) + Raji (human Burkitt's lymphoma cell line) cell lysate at 10

Secondary

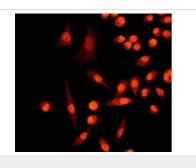
HRP goat anti-rabbit lgG (H+L) at 1/20000 dilution

Predicted band size: 94 kDa **Observed band size:** 100 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

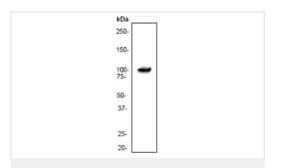
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skin carcinoma tissue labelling STAT6 with unpurified ab32520 at 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-STAT6 antibody [YE361] (ab32520)

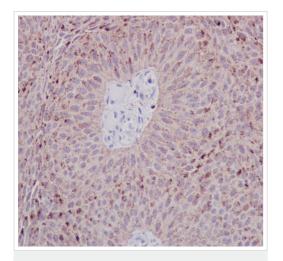
Immunocytochemistry/Immunofluorescence analyis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labelling STAT6 with unpurified ab32520 at 1/100.



Western blot - Anti-STAT6 antibody [YE361] (ab32520)

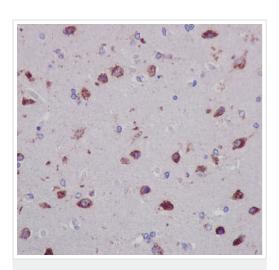
Anti-STAT6 antibody [YE361] (ab32520) at 1/1000 dilution (unpurified) + NIH/3T3 (mouse embyro fibroblast cell line) cell lysate

Predicted band size: 94 kDa **Observed band size:** 100 kDa



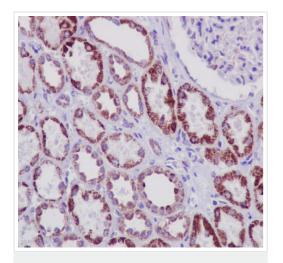
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human transitional cell carcinoma of bladder tissue sections labelling STAT6 with unpurified ab32520 at a dilution of 1/1000. HRP goat anti-rabbit (ab97051) was used at a dilution of 1/500. The antigen retrieval solution was Tris-EDTA buffer, pH 9.0.



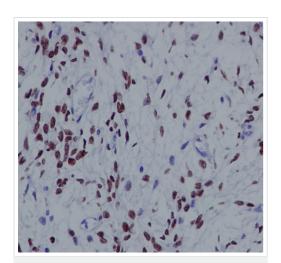
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue sections labelling STAT6 with unpurified ab32520 at a dilution of 1/1000. HRP goat antirabbit (<u>ab97051</u>) was used at a dilution of 1/500. The antigen retrieval solution was Tris-EDTA buffer, pH 9.0.



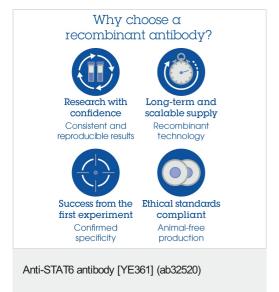
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labelling STAT6 with unpurified ab32520 at a dilution of 1/1000. HRP goat antirabbit (ab97051) was used at a dilution of 1/500. The antigen retrieval solution was Tris-EDTA buffer, pH 9.0.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STAT6 antibody [YE361] (ab32520)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human solitary fibrous tumor tissue sections labelling STAT6 with unpurified ab32520 at a dilution of 1/1000. HRP goat anti-rabbit (ab97051) was used at a dilution of 1/500. The antigen retrieval solution was Tris-EDTA buffer, pH 9.0.



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