

Product datasheet

Anti-TRAF6 antibody [EP592Y] ab40675

KO **VALIDATED** Recombinant RabMAB

★★★★☆ 1 Abreviews 5 References 10 Images

Overview

Product name	Anti-TRAF6 antibody [EP592Y]
Description	Rabbit monoclonal [EP592Y] to TRAF6
Host species	Rabbit
Tested applications	Suitable for: IHC-P, ICC/IF, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human TRAF6 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary.

Positive control

[Purchase matching WB positive control: Recombinant Human TRAF6 protein >](#)

WB: Jurkat, HEK293 and HeLa cell lysates. IHC-P: Human cerebral cortex and mouse kidney tissues. ICC/IF: HeLa cells. This antibody is unsuitable for detecting TRAF6 in tissue lysates.

General notes

Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMab[®] patents](#).

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a [recombinant rabbit monoclonal antibody](#).

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP592Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab40675** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

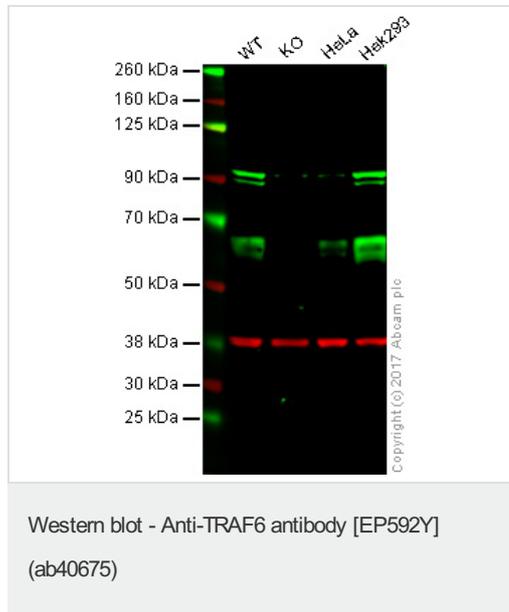
Application	Abreviews	Notes
IHC-P		1/50.
ICC/IF		1/50. For unpurified use at 1/250 - 1/500.
WB	★★★★★	1/5000. Detects a band of approximately 58 kDa (predicted molecular weight: 63 kDa). For unpurified use at 1/2000 - 1/10000.

Target

Function	E3 ubiquitin ligase that, together with UBE2N and UBE2V1, mediates the synthesis of 'Lys-63'-linked-polyubiquitin chains conjugated to proteins, such as IKBKG, AKT1 and AKT2. Also mediates ubiquitination of free/unanchored polyubiquitin chain that leads to MAP3K7 activation. Leads to the activation of NF-kappa-B and JUN. May be essential for the formation of functional osteoclasts. Seems to also play a role in dendritic cells (DCs) maturation and/or activation. Represses c-Myb-mediated transactivation, in B lymphocytes. Adapter protein that seems to play a role in signal transduction initiated via TNF receptor, IL-1 receptor and IL-17 receptor.
Tissue specificity	Expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Belongs to the TNF receptor-associated factor family. A subfamily. Contains 1 MATH domain. Contains 1 RING-type zinc finger. Contains 2 TRAF-type zinc fingers.
Domain	The coiled coil domain mediates homo- and hetero-oligomerization. The MATH/TRAF domain binds to receptor cytoplasmic domains.
Post-translational modifications	Sumoylated on Lys-124, Lys-142 and Lys-453 by SUMO1. Polyubiquitinated on Lys-124; after cell stimulation with IL-1-beta or TGF-beta. This ligand-induced cell stimulation leads to dimerization/oligomerization of TRAF6 molecules, followed by auto-ubiquitination which involves UBE2N and UBE2V1 and leads to TRAF6 activation. This 'Lys-63' site-specific poly-ubiquitination appears to be associated with the activation of signaling molecules. Endogenous autoubiquitination occurs only for the cytoplasmic form.
Cellular localization	Cytoplasm. Cytoplasm > cell cortex. Nucleus. Found in the nuclei of some aggressive B-cell

lymphoma cell lines as well as in the nuclei of both resting and activated T-and B-lymphocytes. Found in punctate nuclear body protein complexes. Ubiquitination may occur in the cytoplasm and sumoylation in the nucleus.

Images



Lane 1: Wild type HAP1 whole cell lysate (20 µg)

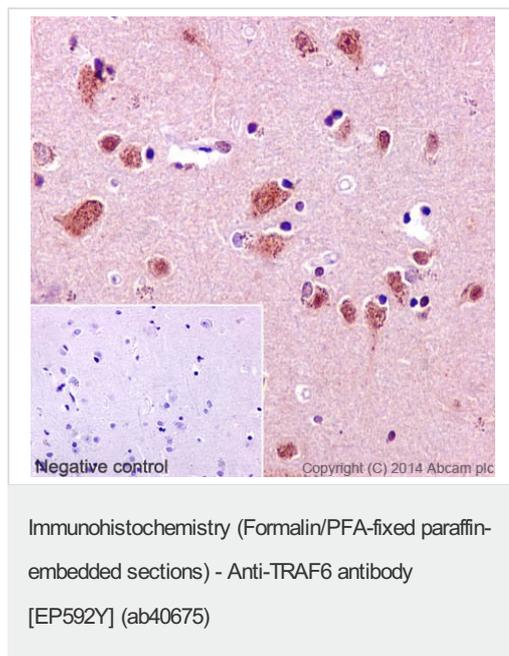
Lane 2: TRAF6 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

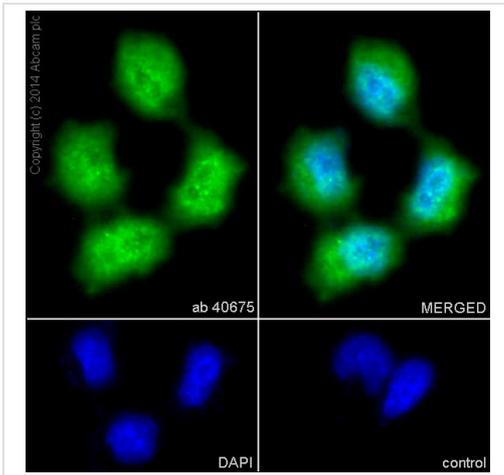
Lane 4: HEK293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab40675 observed at 65 kDa. Red - loading control, ab8245, observed at 37 kDa.

Ab40675 was shown to specifically react with TRAF6 in wild-type cells, along with additional cross-reactive bands as signal was lost in TRAF6 knockout HAP1 cells. Wild-type and TRAF6 knockout samples were subjected to SDS-PAGE. Ab40675 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/500 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.



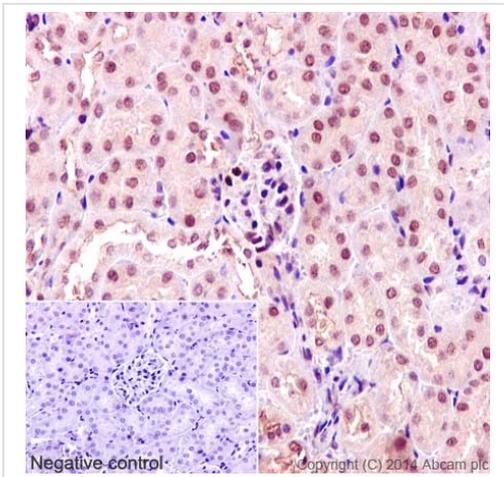
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebral cortex tissue labelling TRAF6 with purified ab40675 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-TRAF6 antibody [EP592Y] (ab40675)

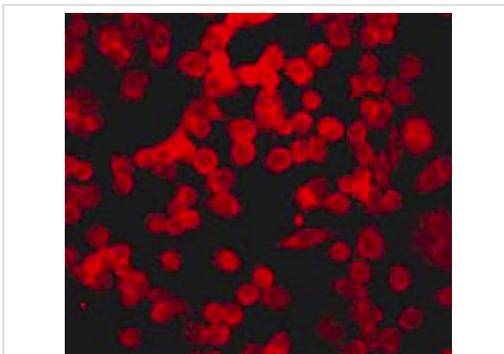
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with purified ab40675 at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. [ab150077](#), an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/50) and secondary antibody, [ab150120](#), an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500).



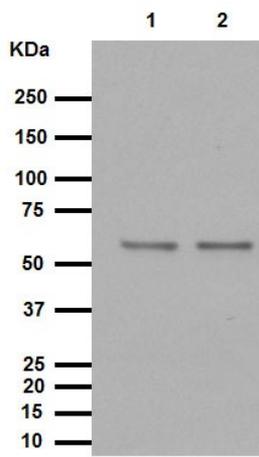
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF6 antibody [EP592Y] (ab40675)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labelling TRAF6 with purified ab40675 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-TRAF6 antibody [EP592Y] (ab40675)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with unpurified ab40675 at 1/250.



Western blot - Anti-TRAF6 antibody [EP592Y]
(ab40675)

All lanes : Anti-TRAF6 antibody [EP592Y] (ab40675) at 1/5000 dilution (purified)

Lane 1 : Jurkat cell lysate

Lane 2 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

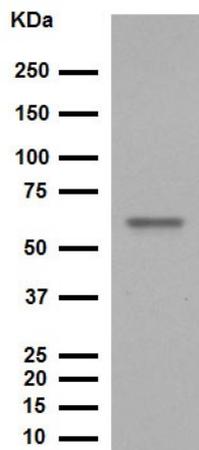
Predicted band size: 63 kDa

Observed band size: 58 kDa

[why is the actual band size different from the predicted?](#)

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-TRAF6 antibody [EP592Y]
(ab40675)

Anti-TRAF6 antibody [EP592Y] (ab40675) at 1/1000 dilution (purified) + HEK293 cell lysate at 20 µg

Secondary

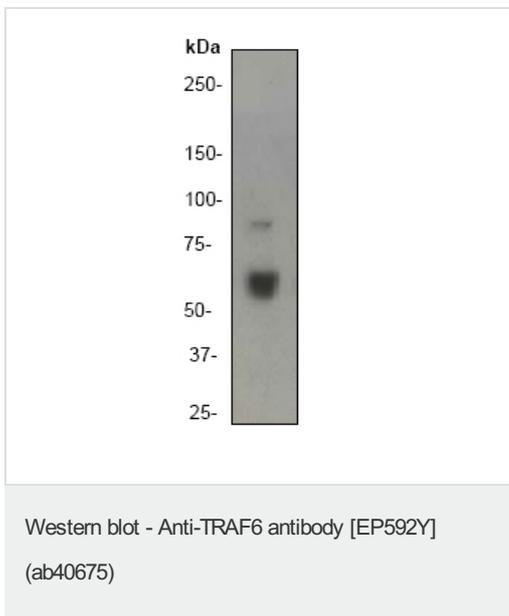
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 63 kDa

Observed band size: 58 kDa [why is the actual band size different from the predicted?](#)

Blocking buffer and concentration: 5% NFDM/TBST.

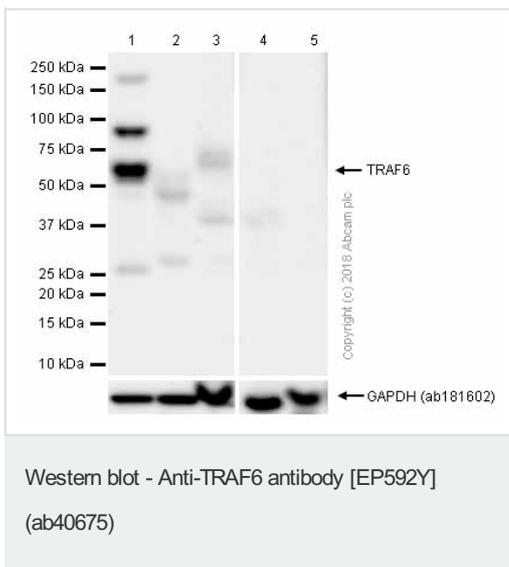
Diluting buffer and concentration: 5% NFDM /TBST.



Anti-TRAF6 antibody [EP592Y] (ab40675) at 1/2000 dilution (unpurified) + 10ug Jurkat cell lysate

Predicted band size: 63 kDa

Observed band size: 58 kDa [why is the actual band size different from the predicted?](#)



All lanes : Anti-TRAF6 antibody [EP592Y] (ab40675) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : Human heart lysates

Lane 3 : Human skeletal muscle lysates

Lane 4 : Mouse skeletal muscle lysates

Lane 5 : Rat skeletal muscle lysates

Lysates/proteins at 15 µg per lane.

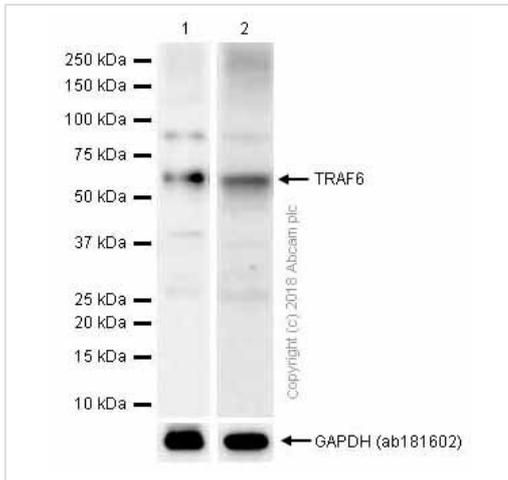
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 63 kDa

Exposure time: 70 seconds

This antibody is unsuitable for detecting tissue lysates.



Western blot - Anti-TRAF6 antibody [EP592Y] (ab40675)

All lanes : Anti-TRAF6 antibody [EP592Y] (ab40675) at 1/1000 dilution

Lane 1 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

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

Predicted band size: 63 kDa

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