

# Anti-TRAF6 antibody [EP592Y] - BSA and Azide free ab227560

KO VALIDATED Recombinant RabMAb

[11 References](#) [7 Images](#)

## Overview

<b>Product name</b>	Anti-TRAF6 antibody [EP592Y] - BSA and Azide free
<b>Description</b>	Rabbit monoclonal [EP592Y] to TRAF6 - BSA and Azide free
<b>Host species</b>	Rabbit
<b>Specificity</b>	This antibody is unsuitable for detecting tissue lysates in WB application.
<b>Tested applications</b>	<b>Suitable for:</b> WB, ICC/IF, IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HAP1, Daudi, Jurkat, HEK293 and HeLa cell lysates. IHC-P: Human cerebral cortex and mouse kidney tissues. ICC/IF: HeLa cells.
<b>General notes</b>	<p>ab227560 is the carrier-free version of <a href="#">ab40675</a>.</p> <p>Our <b>carrier-free</b> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with &lt;1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.</p>

## Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C. Do Not Freeze.
<b>Storage buffer</b>	pH: 7.20

	Constituent: PBS
<b>Carrier free</b>	Yes
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EP592Y
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab227560 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
<b>WB</b>		Use at an assay dependent concentration. Detects a band of approximately 58 kDa (predicted molecular weight: 63 kDa).
<b>ICC/IF</b>		Use at an assay dependent concentration.
<b>IHC-P</b>		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

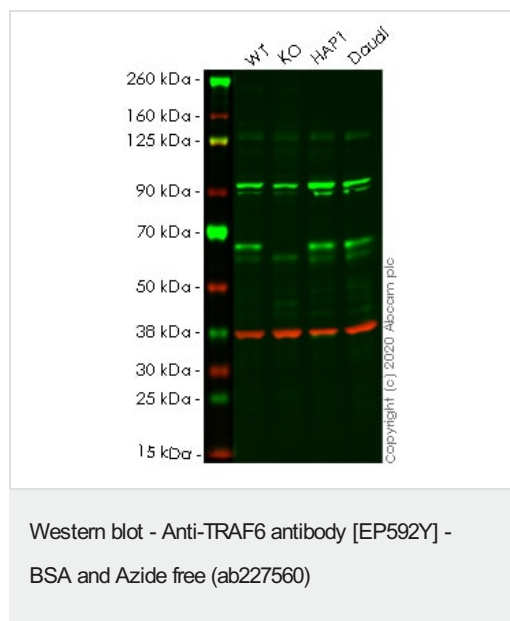
## Target

<b>Function</b>	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.
<b>Tissue specificity</b>	Expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
<b>Pathway</b>	Protein modification; protein ubiquitination.
<b>Sequence similarities</b>	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.
<b>Domain</b>	The coiled coil domain mediates homo- and hetero-oligomerization. The MATH/TRAF domain binds to receptor cytoplasmic domains.
<b>Post-translational modifications</b>	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



**All lanes :** Anti-TRAF6 antibody [EP592Y] ([ab40675](#)) at 1/500 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** TRAF6 knockout HeLa cell lysate

**Lane 3 :** HAP1 cell lysate

**Lane 4 :** Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

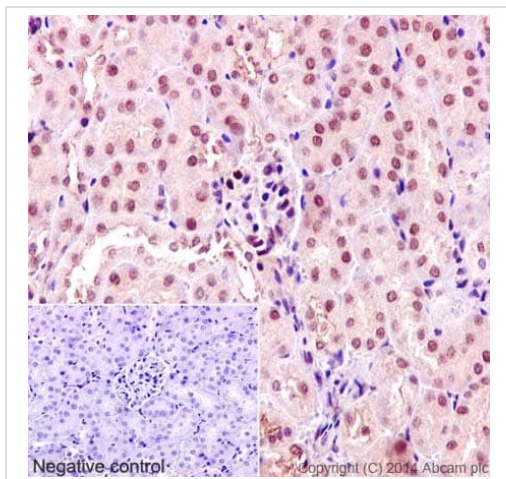
**Predicted band size:** 63 kDa

**Observed band size:** 65 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab40675](#)).

**Lanes 1- 4:** Merged signal (red and green). Green - [ab40675](#) observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

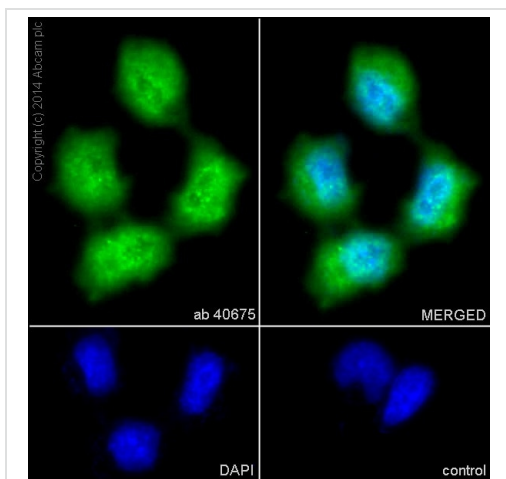
[ab40675](#) was shown to react with TRAF6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line [ab266009](#) (knockout cell lysate [ab257760](#)) was used. Wild-type HeLa and TRAF6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. [ab40675](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 500 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-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40675**).

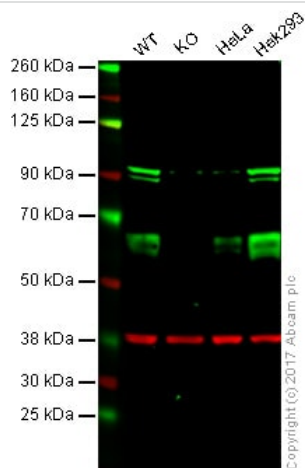


Immunocytochemistry/ Immunofluorescence - Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

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<sup>®</sup> 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<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40675**).



Western blot - Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

This WB data was generated using the same anti-TRAF6 antibody clone, EP592Y, in a different buffer formulation (cat# [ab40675](#)).

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

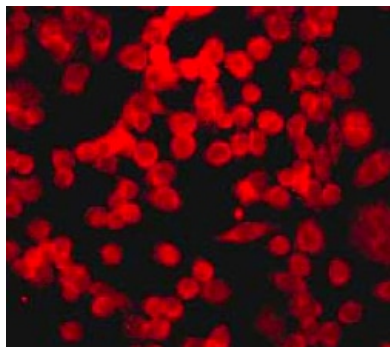
Lane 2: TRAF6 knockout HAP1 whole cell lysate (20 µg)

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.

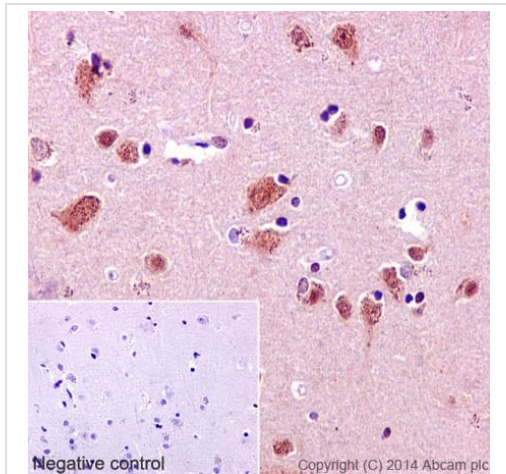
Ab40675 was shown to specifically react with TRAF6 in wild-type cells, along with additional cross-reactive bands as signal was lost in TRAF6 knockout HAP1 cells. Wild-type and TRAF6 knockout samples were subjected to SDS-PAGE. Ab40675 and [ab8245](#) (Mouse anti GAPDH loading control) were incubated 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.



Immunocytochemistry/ Immunofluorescence - Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with unpurified [ab40675](#) at 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab40675](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

This IHC data was generated using the same anti-TRAF6 antibody clone, EP592Y, in a different buffer formulation (cat# [ab40675](#)).

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.

#### Why choose a recombinant antibody?



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Recombinant technology



**Success from the first experiment**  
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Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560)

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