abcam

ab218575

Anti-TRAF6 antibody [EP591Y] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP591Y
Purification technique	Affinity purification Protein A
Specificity	This antibody is unsuitable for detecting TRAF6 in tissue lysates.
Concentration	0.941 - 1.053 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Rat, Human, Zebrafish
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376- Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

Target data

See full target information TRAF6

Function

E3 ubiquitin ligase that, together with UBE2N and UBE2V1, mediates the synthesis of 'Lys-63'-linked-polyubiquitin chains conjugated to proteins, such as ECSIT, IKBKG, IRAK1, AKT1 and AKT2 (PubMed:11057907, PubMed:18347055, PubMed:19465916, PubMed:19713527, PubMed:27746020, PubMed:31620128). Also mediates ubiquitination of free/unanchored polyubiquitin chain that leads to MAP3K7 activation (PubMed:19675569). Leads to the activation of NF-kappa-B and JUN (PubMed:16378096, PubMed:17135271, PubMed:17703191). Seems to also play a role in dendritic cells (DCs) maturation and/or activation (By similarity). Represses c-Myb-mediated transactivation, in B-lymphocytes (PubMed:18093978, PubMed:18758450). Adapter protein that seems to play a role in signal transduction initiated via TNF receptor, IL-1 receptor and IL-17 receptor (PubMed:12140561, PubMed:19825828, PubMed:8837778). Regulates osteoclast differentiation by mediating the activation of adapter protein complex 1 (AP-1) and NF-kappa-B, in response to RANK-L stimulation (By similarity). Together with MAP3K8, mediates CD40 signals that activate ERK in B-cells and macrophages, and thus may play a role in the regulation of immunoglobulin production (By similarity). Acts as a regulator of the JNK and NF-kappa-B signaling pathways by initiating assembly of heterotypic 'Lys-63'-/'Lys-48'-linked branched ubiquitin chains that are then recognized by TAB2: TRAF6 catalyzes initial 'Lys-63'-linked-

polyubiquitin chains that are then branched via 'Lys-48'-linked polyubiquitin by HUWE1 (PubMed:27746020). 'Lys-63'-/'Lys-48'-linked branched ubiquitin chains protect 'Lys-63'-linkages from CYLD deubiquitination (PubMed:27746020). Participates also in the TCR signaling by ubiquitinating LAT (PubMed:23514740, PubMed:25907557).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab218575 is the carrier-free version of ab33915.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	TRAF6 also known as TNF receptor-associated factor 6 is an E3 ubiquitin ligase that is part of the TRAF family. It has a molecular mass of approximately 60 kDa. TRAF6 is ubiquitously expressed in many types of tissues and cells including immune cells like macrophages and dendritic cells. Its primary role is to mediate signal transduction from members of the TNF receptor and Toll-like receptor (TLR) families which it achieves through polyubiquitination of target proteins. This process facilitates the recruitment of downstream signaling components necessary for cellular responses.
Biological function summary	TRAF6 plays a critical role in regulating immune and inflammatory responses. It acts within a complex often associating with other signaling proteins such as IRAK1 and TAK1 to activate downstream kinases like IKK and JNK. These signaling pathways lead to the activation of transcription factors such as NF-kB and AP-1 which regulate the expression of genes involved in inflammation and immune responses. TRAF6 is an important modulator of adaptive and innate immunity influencing both the maturation of immune cells and the body's response to infection.
Pathways	TRAF6 significantly contributes to the NF-kB and MAPK signaling pathways. In the NF-kB pathway TRAF6 mediates signal transduction following TLR engagement interacting with proteins such as MyD88 and RIP1 to drive inflammatory cytokine production. In the MAPK pathway it associates predominantly with TAK1 to facilitate the phosphorylation and activation of downstream kinases. These pathways are essential for transmitting signals from cell surface receptors to the nucleus controlling the magnitude of immune signaling and cellular stress responses.
Associated diseases and disorders	TRAF6 has a significant association with autoimmune diseases and certain cancers. In autoimmune disorders aberrant activation of TRAF6 can lead to prolonged inflammation and detrimental immune responses. For cancers TRAF6 is linked to tumor progression and metastasis acting alongside proteins like IRAK1 and RIP1 which can further promote malignancy through chronic inflammation and immune evasion. Understanding TRAF6's role in these diseases provides insights that could lead to targeted therapeutic strategies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

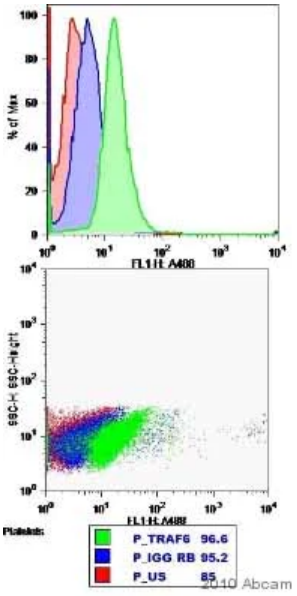
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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7 product images

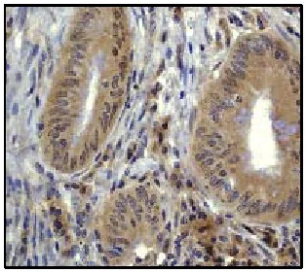


This image is courtesy of an anonymous customer review.

Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Flow Cytometry (Intracellular) staining using rabbit Anti-TRAF6 antibody

ab33915 staining TRAF6 in Human platelet cells by intracellular flow cytometry.Cells were fixed in paraformaldehyde and permeabilized using 0.1% Triton-X-100 in 2% BSA for 15 minutes. Primary antibody used at a 1/200 dilution and incubated for 16 hours at 4°C. The secondary antibody used was an Alexa Fluor® 488 conjugated chicken anti-rabbit IgG (H+L) at a 1/500 dilution.
P : permeabilized US : Unstained (Red Peak) IGG RB : IgG Rabbit (Blue Peak) TRAF6 Ab (Green Peak)This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab33915).

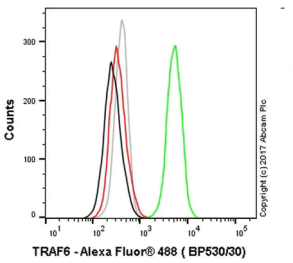


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TRAF6 antibody

This IHC data was generated using the same anti-TRAF6 antibody clone, EP591Y, in a different buffer formulation (cat# ab33915).
Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma using anti-TRAF6 (ab33915)

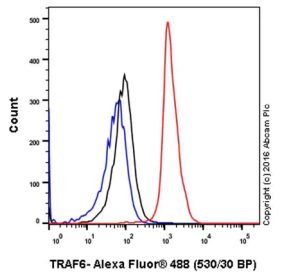
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Flow Cytometry (Intracellular) staining using rabbit Anti-TRAF6 antibody

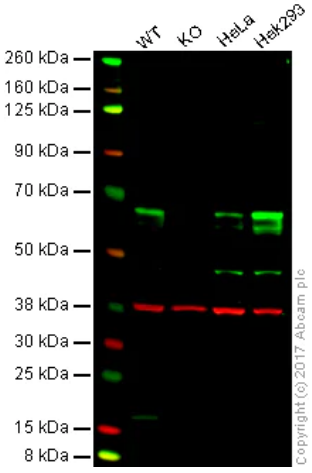
Overlay histogram showing HAP1 wildtype (green line) and HAP1-TRAF6 knockout cells (red line) stained with ab33915. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab33915, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) preadsorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-TRAF6 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab33915).



Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Flow Cytometry (Intracellular) staining using rabbit Anti-TRAF6 antibody

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling TRAF6 with purified ab33915 at 1/240 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab33915).



Western blot - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Western blot staining using rabbit Anti-TRAF6 antibody

This WB data was generated using the same anti-TRAF6 antibody clone, EP591Y, in a different buffer formulation (cat# ab33915).
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)

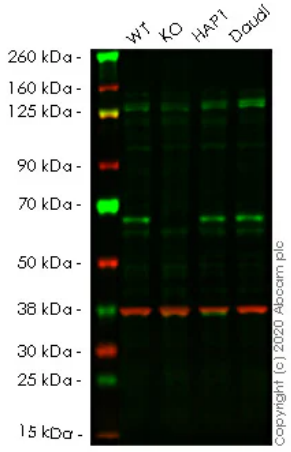
Lanes 1 - 4: Merged signal (red and green). Green - ab33915 observed at 65 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab33915 was shown to specifically react with TRAF6 in wild-type cells as signal was lost in TRAF6 knockout HAP1 cells. Wild-type and TRAF6 knockout samples were subjected to SDS-PAGE. ab33915 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 2000 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

All lanes:

Western blot - Anti-TRAF6 antibody [EP591Y] (ab33915)

Predicted band size: 60 kDa



Western blot - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Western blot staining using rabbit Anti-TRAF6 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab33915).
Lanes 1- 4: Merged signal (red and green). Green - ab33915 observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab33915 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. ab33915 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 2000 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.

All lanes:
Western blot - Anti-TRAF6 antibody [EP591Y] (ab33915) at 1/2000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human TRAF6 knockout HeLa cell lysate (ab257760) at 20 µg

Lane 3:
HAP1 cell lysate at 20 µg

Lane 4:
Daudi cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 65 kDa

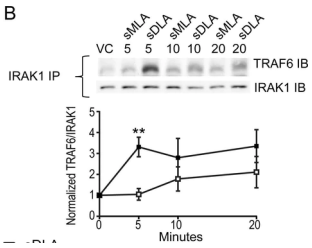


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-TRAF6 antibody [EP591Y] - BSA and Azide free (ab218575)

TRAF6 Western blot staining using rabbit Anti-TRAF6 antibody

TRAF6 western blot using anti-TRAF6 antibody [EP591Y] - BSA and Azide free ab218575. Publication image and figure legend from Casella, C. R. & Mitchell, T. C., 2013, PLoS One, PubMed 23638128.

ab218575 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab218575 please see the product overview.

Reduced MyD88-associated signaling by sMLA.Mouse BMDC were treated with 100 ng/ml sMLA or sDLA for the indicated times, lysed and either immunoblots (A,C) or immunoprecipitation (B) were performed on the lysates. A) Immunoblots were probed for IRAK1 and β-actin. A representative gel is depicted with a graph that shows the mean +/- SEM from 5 experiments in which IRAK1 levels were normalized to β-actin levels and vehicle control (VC, measured at 15 min.). B) Lysates were immunoprecipitated with IRAK1 antibodies and immunoblotted with antibodies for TRAF6 and IRAK1. The graph shows mean +/- SEM from 8 experiments with at least 5 data points per time point. TRAF6 levels were normalized based on IRAK1 levels and VC (5 min. time point). C) Left, Immunoblots were probed for phosphoERK 1/2 (Thr202/Tyr204), stripped and re-probed for ERK total, then β-actin. Shown are the mean +/- SEM from 5 experiments with levels of pERK1/2 normalized to ERK total and VC (15 min. time point). Right, immunoblots were probed for IκBα stripped and probed for β-actin. Shown are the mean +/- SEM from 4 experiments with levels of IκBα normalized to β-actin and VC (5 min. time point). Two way ANOVAs with Sidak's multiple comparisons were performed for A and C and two tailed paired T test for B. Asterisks indicate a significant differences between sMLA and sDLA at the indicated time points with * $p<0.05$, ** $p<0.01$ and, **** $p<0.001$, respectively.

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