Overview

Product name: Anti-TRAF6 antibody [EP591Y]
Description: Rabbit monoclonal [EP591Y] to TRAF6
Host species: Rabbit
Tested applications: Suitable for: WB, Flow Cyt, IHC-P
Unsuitable for: IP
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Zebrafish
Immunogen: Synthetic peptide within Human TRAF6 aa 50-150 (N terminal). The exact sequence is proprietary.
(Peptide available as ab183540)
Positive control: Jurkat and HAP1 whole cell lysate. Human colon adenocarcinoma. 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
This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid
Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 49% PBS, 50% Glycerol, 0.05% BSA
Purity: IgG fraction
Clonality: Monoclonal
Clone number: EP591Y
Isotype: IgG
**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**
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 agressive 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.
Western blot - Anti-TRAF6 antibody [EP591Y] (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.

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.
Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma using anti-TRAF6 (ab33915)

ab33915 staining TRAF6 in Human platelet cells by 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)
Western blot - Anti-TRAF6 antibody [EP591Y] (ab33915) at 1/10000 dilution + Jurkat cell lysate

Predicted band size: 58 kDa

Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling TRAF6 with purified ab33915 at 1/240 dilution (10µg/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.
Western blot - Anti-TRAF6 antibody [EP591Y] (ab33915)

**All lanes:** Anti-TRAF6 antibody [EP591Y] (ab33915) 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:** 58 kDa

This antibody is unsuitable for detecting tissue lysates.

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

**All lanes:** Anti-TRAF6 antibody [EP591Y] (ab33915) 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:** 58 kDa
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