


### Anti-TRAF6 antibody [EP591Y] ab33915

敲除验证
重组
RabMAb

★★★★☆
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#### 概述

<b>产品名称</b>	Anti-TRAF6抗体[EP591Y]
<b>描述</b>	兔单克隆抗体[EP591Y] to TRAF6
<b>宿主</b>	Rabbit
<b>特异性</b>	This antibody is unsuitable for detecting TRAF6 in tissue lysates.
<b>经测试应用</b>	<b>适用于:</b> Flow Cyt (Intra), WB, IHC-P <b>不适用于:</b> IP
<b>种属反应性</b>	<b>与反应:</b> Mouse, Rat, Human <b>预测可用于:</b> Zebrafish 
<b>免疫原</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>阳性对照</b>	HeLa, Daudi, Jurkat and HAP1 whole cell lysate. Human colon adenocarcinoma.
<b>常规说明</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 性能

<b>形式</b>	Liquid
<b>存放说明</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
<b>存储溶液</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
<b>纯度</b>	Protein A purified
<b>克隆</b>	单克隆

克隆编号 EP591Y

同种型 IgG

## 应用

**The Abpromise guarantee** **Abpromise™** 承诺保证使用 ab33915 于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

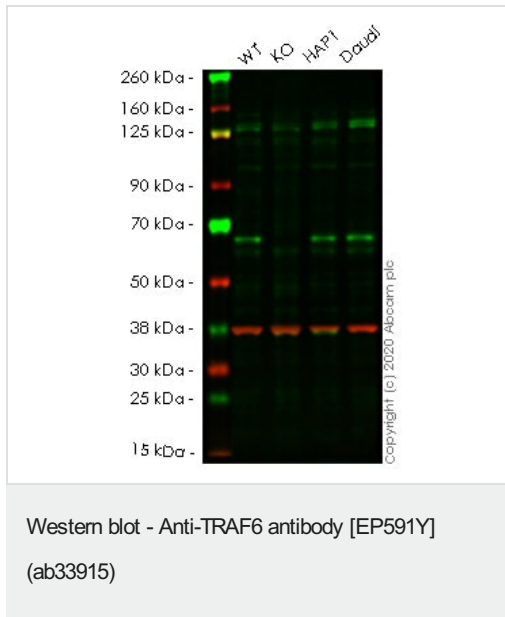
应用	Ab评论	说明
Flow Cyt (Intra)		1/50. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (8)	1/2000 - 1/10000. Predicted molecular weight: 58 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

应用说明 Is unsuitable for IP.

## 靶标

功能	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.
组织特异性	Expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
通路	Protein modification; protein ubiquitination.
序列相似性	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.
结构域	The coiled coil domain mediates homo- and hetero-oligomerization. The MATH/TRAF domain binds to receptor cytoplasmic domains.
翻译后修饰	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.
细胞定位	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

图片



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

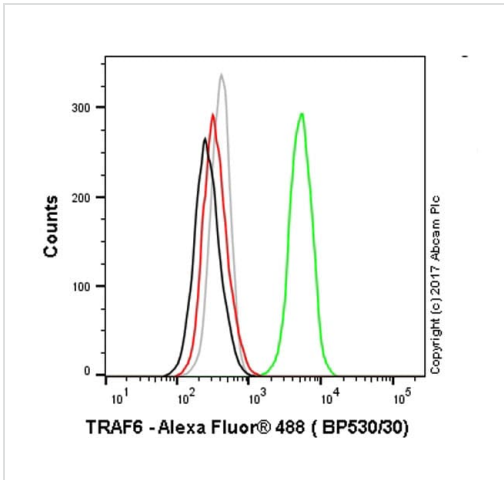
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 58 kDa  
**Observed band size:** 65 kDa

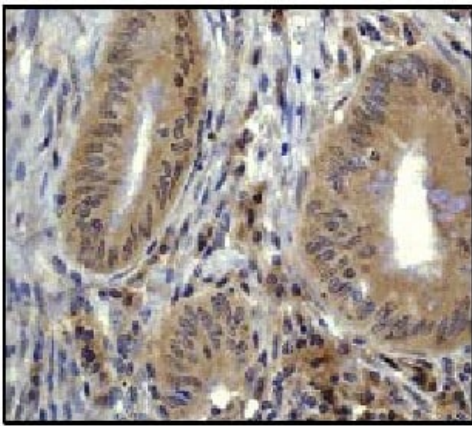
**Lanes 1-4:** Merged signal (red and green). Green - ab33915 observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

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.



Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] (ab33915)

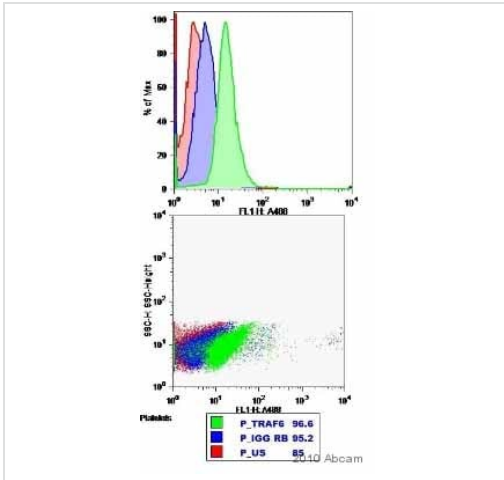
Overlay histogram showing HAP1 wildtype (green line) and HAP1-TRAF6 knockout cells (red line) stained with ab33915. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab33915, 0.1 µg/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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF6 antibody [EP591Y] (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.

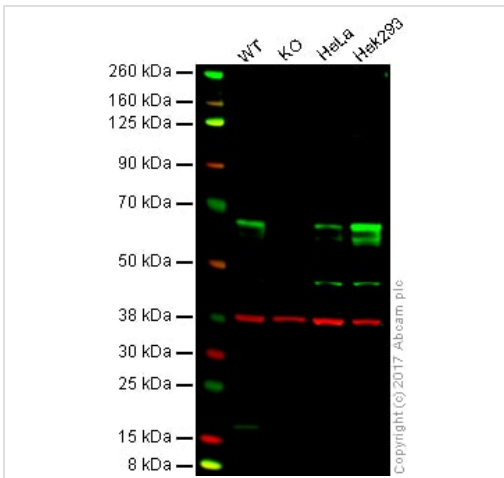


Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] (ab33915)

Image courtesy of an anonymous Abreview.

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)



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

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

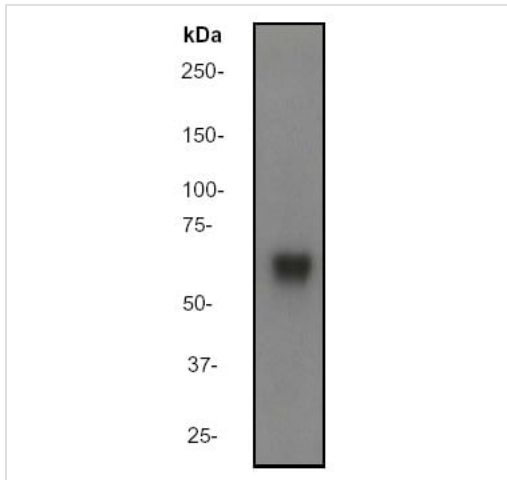
**Lane 2:** TRAF6 knockout HAP1 whole cell lysate (20 µg)

**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.

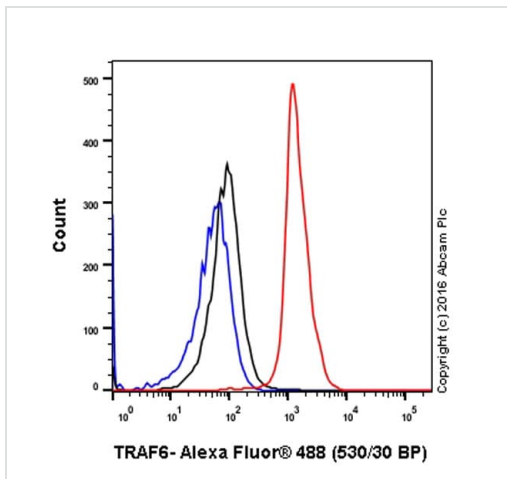
Ab33915 was shown to specifically react with TRAF6 in wild-type cells as signal was lost in TRAF6 knockout HAP1 cells. Wild-type and TRAF6 knockout samples were subjected to SDS-PAGE. Ab33915 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 2000 dilution and 1/10000 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



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

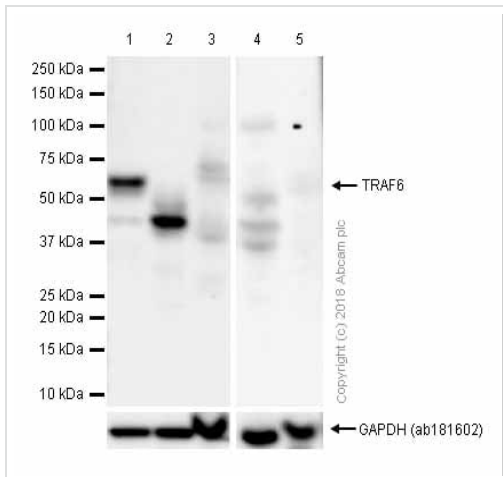
Anti-TRAF6 antibody [EP591Y] (ab33915) at 1/10000 dilution + Jurkat cell lysate

**Predicted band size: 58 kDa**



Flow Cytometry (Intracellular) - Anti-TRAF6 antibody [EP591Y] (ab33915)

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.



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

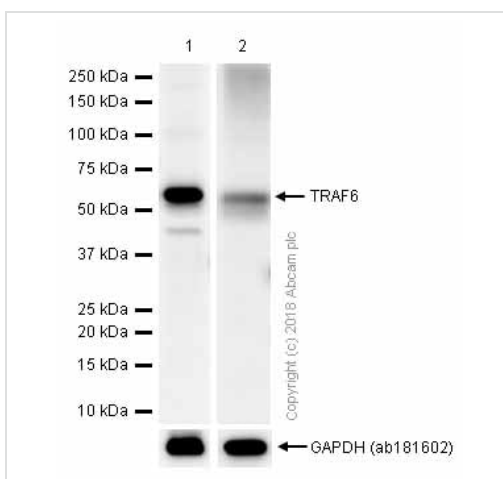
**Lane 5** : Rat skeletal muscle lysates

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



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

Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results

**Long-term and scalable supply**  
Recombinant technology

**Success from the first experiment**  
Confirmed specificity

**Ethical standards compliant**  
Animal-free production

Anti-TRAF6 antibody [EP591Y] (ab33915)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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