# abcam

# Product datasheet

# Anti-TRAF6 antibody [EP592Y] ab40675





重组 RabMAb

★★★★★ 1 Abreviews 31 References 11 图像

概述

产品名称 Anti-TRAF6抗体[EP592Y]

描述 兔单克隆抗体[EP592Y] to TRAF6

宿主 Rabbit

特异性 This antibody is unsuitable for detecting tissue lysates in WB application.

经测试应用 适用于: IHC-P, ICC/IF, WB 种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: HAP1, Daudi, Jurkat, HEK293 and HeLa cell lysates. IHC-P: Human cerebral cortex and

mouse kidney tissues. ICC/IF: HeLa cells.

常规说明 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 production

For more information see here.

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

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

纯度 Protein A purified

克隆 单克隆 克隆编号 **EP592Y** 

同种型 lgG

#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab40675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 <b>用</b>	Ab评论	说明
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/50. For unpurified use at 1/250 - 1/500.
WB	<b>★★★★☆ (1)</b>	1/5000. Detects a band of approximately 58 kDa (predicted molecular weight: 63 kDa).  For unpurified use at 1/2000 - 1/10000.  This antibody is unsuitable for detecting tissue lysates.

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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 agressive B-cell

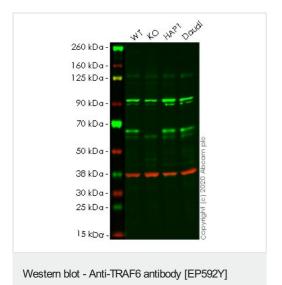
lymphoma cell lines as well as in the nuclei of both resting and activated T-and B-lymphocytes. Found in punctate nuclear body protein complexes. Ubiquitination may occur in the cytoplasm and

sumoylation in the nucleus.

细胞化型

2

(ab40675)



**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

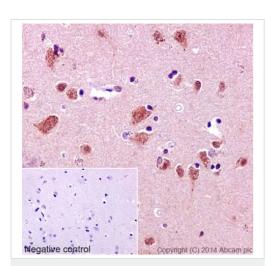
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63 kDa
Observed band size: 65 kDa

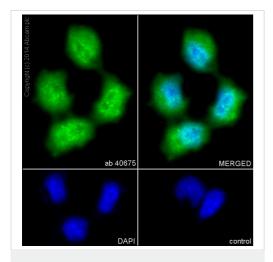
**Lanes 1-4:** Merged signal (red and green). Green - ab40675 observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG 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 paraffinembedded sections) - Anti-TRAF6 antibody
[EP592Y] (ab40675)

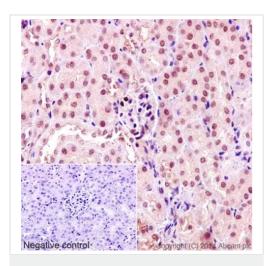
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebral cortex tissue labelling TRAF6 with purified ab40675 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit 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)

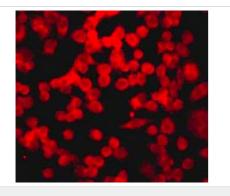
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with purified ab40675 at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary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

Control: primary antibody (1/50) and secondary antibody, <u>ab150120</u>, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).



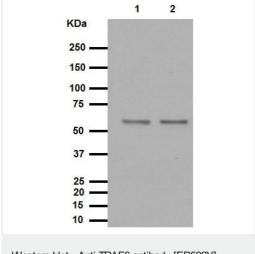
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRAF6 antibody
[EP592Y] (ab40675)

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. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit lgG (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.

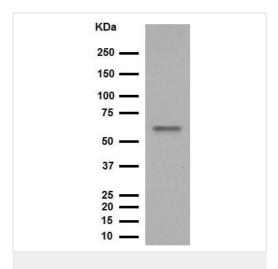
# Secondary

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

Predicted band size: 63 kDa Observed band size: 58 kDa

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

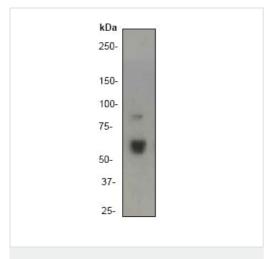
#### Secondary

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

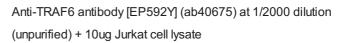
Predicted band size: 63 kDa Observed band size: 58 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

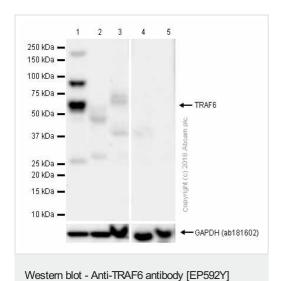
Diluting buffer and concentration: 5% NFDM /TBST.



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



**Predicted band size:** 63 kDa **Observed band size:** 58 kDa



(ab40675)

**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.

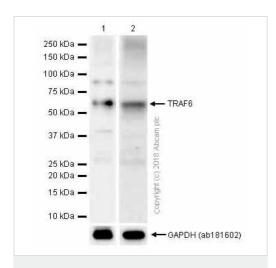
# Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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 virusinduced tumor macrophage) whole cell lysates

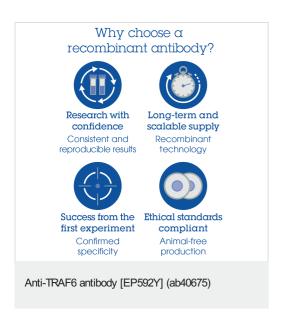
Lane 2: PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lysates/proteins at 15 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 63 kDa



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