# abcam

# **Product datasheet**

# Anti-TRAF6 antibody [EP592Y] - BSA and Azide free ab227560 **敲除 验证** 重组 RabMAb

<u>11 References</u> 7 图像

| 概述                   |   |  |
|----------------------|---|--|
| 产 <b>品名称</b>         | Anti-TRAF6 <b>抗体</b> [EP592Y] - BSA and Azide free  |  |
| 描述                   | 兔单克隆抗体[EP592Y] to TRAF6 - BSA and Azide free  |  |
| 宿主                   | Rabbit  |  |
| 特异性                  | This antibody is unsuitable for detecting tissue lysates in WB application.   |  |
| 经测试应 <b>用</b>        | 适用于: WB, ICC/IF, IHC-P  |  |
| <b>种属反</b> 应性        | 与反应: Mouse, Rat, Human  |  |
| 免疫原                  | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.   |  |
| <b>阳性</b> 对 <b>照</b> | WB: HAP1, Daudi, Jurkat, HEK293 and HeLa cell lysates. IHC-P: Human cerebral cortex and mouse kidney tissues. ICC/IF: HeLa cells.   |  |
| <b>常</b> 规说 <b>明</b> | ab227560 is the carrier-free version of <u>ab40675</u> .  |  |
|                      | Our <b><u>carrier-free</u></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.                                       |  |
|                      | 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 <b>conjugation kits</b> 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.   |  |
|                      | This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. $Maxpar^{®}$ is a trademark of Fluidigm Canada Inc.   |  |
| 性能                   |   |  |
| 形式                   | Liquid  |  |
| 存 <b>放</b> 说明        | Shipped at 4°C. Store at +4°C. Do Not Freeze.   |  |
| 存储溶液                 | pH: 7.20  |  |
|                      | Constituent: PBS  |  |

| 无载体          | 是                  |
|--------------|--------------------|
| 纯 <b>度</b>   | Protein A purified |
| 克隆           | 单 <b>克隆</b>        |
| <b>克隆</b> 编号 | EP592Y             |
| 同种型          | lgG                |

应用

## The Abpromise guarantee

### Abpromise™承诺保证使用ab227560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。

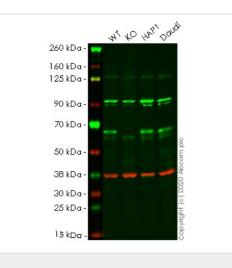
| 应 <b>用</b> | Ab评论 | 说明  |
|------------|------|---|
| WB         |      | Use at an assay dependent concentration. Detects a band of approximately 58 kDa (predicted molecular weight: 63 kDa).                                       |
| ICC/IF     |      | Use at an assay dependent concentration.  |
| IHC-P      |      | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

**靶**标

| 功能            | E3 ubiquitin ligase that, together with UBE2N and UBE2V1, mediates the synthesis of 'Lys-63'-<br>linked-polyubiquitin chains conjugated to proteins, such as IKBKG, AKT1 and AKT2. Also<br>mediates ubiquitination of free/unanchored polyubiquitin chain that leads to MAP3K7 activation.<br>Leads to the activation of NF-kappa-B and JUN. May be essential for the formation of functional<br>osteoclasts. Seems to also play a role in dendritic cells (DCs) maturation and/or activation.<br>Represses c-Myb-mediated transactivation, in B lymphocytes. Adapter protein that seems to play<br>a role in signal transduction initiated via TNF receptor, IL-1 receptor and IL-17 receptor. |
|---------------|---|
| 组织 <b>特异性</b> | 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.<br>Contains 1 MATH domain.<br>Contains 1 RING-type zinc finger.<br>Contains 2 TRAF-type zinc fingers.  |
| 结 <b>构域</b>   | The coiled coil domain mediates homo- and hetero-oligomerization.<br>The MATH/TRAF domain binds to receptor cytoplasmic domains.  |
| <b>翻译后修</b> 饰 | Sumoylated on Lys-124, Lys-142 and Lys-453 by SUMO1.<br>Polyubiquitinated on Lys-124; after cell stimulation with IL-1-beta or TGF-beta. This ligand-<br>induced cell stimulation leads to dimerization/oligomerization of TRAF6 molecules, followed by<br>auto-ubiquitination which involves UBE2N and UBE2V1 and leads to TRAF6 activation. This 'Lys-<br>63' site-specific poly-ubiquitination appears to be associated with the activation of signaling<br>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.

#### 图片



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

All lanes : Anti-TRAF6 antibody [EP592Y] (<u>ab40675</u>) 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.

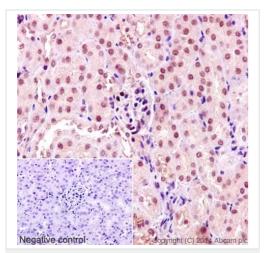
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (<u>ab40675</u>).

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

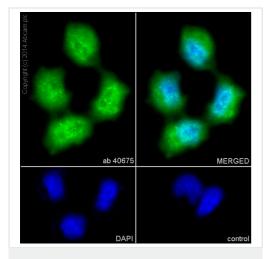
<u>ab40675</u> was shown to react with TRAF6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line <u>ab266009</u> (knockout cell lysate <u>ab257760</u>) was used. Wild-type HeLa and TRAF6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. <u>ab40675</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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<sup>®</sup>800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup>680RD) preadsorbed (<u>ab216776</u>) 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] - BSA and Azide free (ab227560)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labelling TRAF6 with purified <u>ab40675</u> at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <u>ab97051</u>, 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.

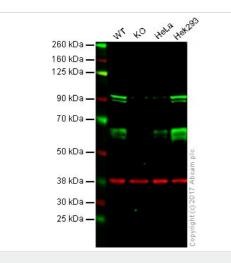
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40675**).



Immunocytochemistry/ Immunofluorescence - Anti-TRAF6 antibody [EP592Y] - BSA and Azide free (ab227560) Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with purified <u>ab40675</u> at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <u>ab150077</u>, 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).

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



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

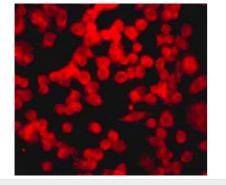
This WB data was generated using the same anti-TRAF6 antibody clone, EP592Y, in a different buffer formulation (cat# **ab40675**). Lane 1: Wild type HAP1 whole cell lysate (20 µg) Lane 2: TRAF6 knockout HAP1 whole cell lysate (20 µg) Lane 3: HeLa whole cell lysate (20 µg) Lane 4: HEK293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab40675</u> observed at 65 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

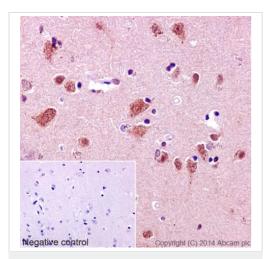
Ab40675 was shown to specifically react with TRAF6 in wild-type cells, along with additional cross-reactive bands as signal was lost in TRAF6 knockout HAP1 cells. Wild-type and TRAF6 knockout samples were subjected to SDS-PAGE. Ab40675 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling TRAF6 with unpurified <u>ab40675</u> 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**).



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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

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