abcam

Product datasheet

Anti-TRAF2 antibody [EPR6048] ab126758

KO VALIDATED Recombinant RabMAb

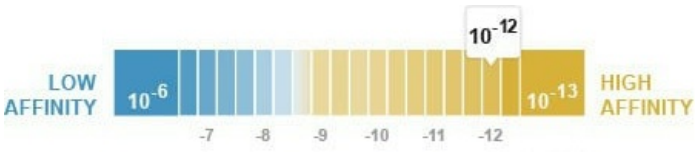
23 References 14 Images

Overview

Product name	Anti-TRAF2 antibody [EPR6048]
Description	Rabbit monoclonal [EPR6048] to TRAF2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Rat, Human **Predicted to work with:** Mouse
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, PC12, MOLT4, HeLa, and 293T cell lysates. ICC: HeLa cells. IHC-P: Human kidney tissue. Flow Cyt (intra): HeLa cells.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Dissociation constant (K_D)	$K_D = 1.70 \times 10^{-12}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6048
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab126758 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

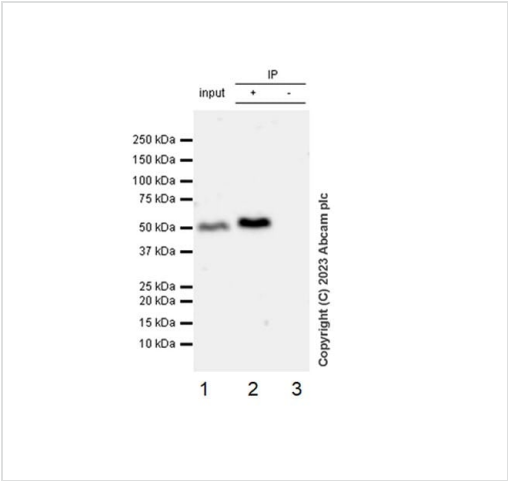
Application	Abreviews	Notes
Flow Cyt (Intra)		1/120. For unpurified, use 1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 53 kDa (predicted molecular weight: 55 kDa).
IP		1/40.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		1/100.

Target

Function	Regulates activation of NF-kappa-B and JNK and plays a central role in the regulation of cell survival and apoptosis. Required for normal antibody isotype switching from IgM to IgG. Has E3 ubiquitin-protein ligase activity and promotes 'Lys-63'-linked ubiquitination of target proteins, such as BIRC3, RIPK1 and TICAM1. Is an essential constituent of several E3 ubiquitin-protein ligase complexes, where it promotes the ubiquitination of target proteins by bringing them into contact with other E3 ubiquitin ligases. Regulates BIRC2 and BIRC3 protein levels by inhibiting their autoubiquitination and subsequent degradation; this does not depend on the TRAF2 RING-type zinc finger domain.
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.

	The RING-type zinc finger domain is essential for E3 ubiquitin-protein ligase activity. It is not essential for the stabilization of BIRC2, or for the ubiquitination of RIPK1 in response to TNFR1 signaling.
Post-translational modifications	Phosphorylated at several serine residues within the first 128 amino acid residues. Phosphorylated at Thr-117 in response to signaling via TNF and TNFRSF1A. Phosphorylation at Thr-117 is required for 'Lys-63'-linked polyubiquitination, but not for 'Lys-48'-linked polyubiquitination. Phosphorylation at Thr-117 is important for interaction with IKKA and IKKB, activation of IKK and subsequent activation of NF-kappa-B. Undergoes both 'Lys-48'-linked and 'Lys-63'-linked polyubiquitination. Polyubiquitinated via 'Lys-63'-linked ubiquitin in response to TNF signaling; this requires prior phosphorylation at Thr-117. 'Lys-63'-linked polyubiquitination promotes TRAF2-mediated activation of NF-kappa-B. Can be polyubiquitinated at several Lys residues via 'Lys-48'-linked ubiquitin chains in response to TNF signaling, leading to proteasomal degradation. Autoubiquitinated, leading to its subsequent proteasomal degradation. Polyubiquitinated by BIRC2 and SIAH2, leading to its subsequent proteasomal degradation. Deubiquitinated by CYLD, a protease that specifically cleaves 'Lys-63'-linked polyubiquitin chains.
Cellular localization	Cytoplasm.

Images



Immunoprecipitation - Anti-TRAF2 antibody [EPR6048] (ab126758)

TRAF2 was immunoprecipitated from HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate with ab126758 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab126758 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate, 10µg

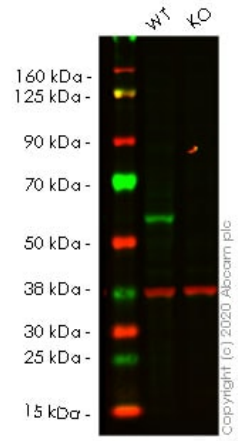
Lane 2: HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab126758 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Observed MV: 55kDa.

Exposure time: 58 seconds.



Western blot - Anti-TRAF2 antibody [EPR6048] (ab126758)

All lanes : Anti-TRAF2 antibody [EPR6048] (ab126758) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : TRAF2 knockout HEK-293T cell lysate

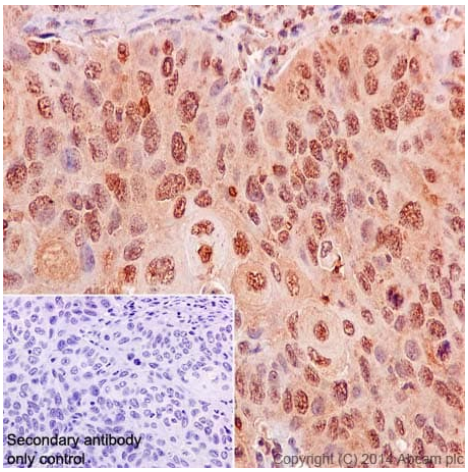
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 55 kDa

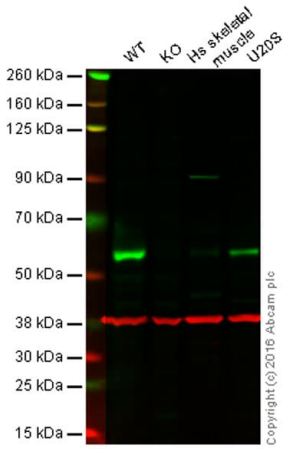
Lanes 1- 2: Merged signal (red and green). Green - ab126758 observed at 55 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab126758 was shown to react with TRAF2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266060** (knockout cell lysate **ab257759**) was used. Wild-type HEK-293T and TRAF2 knockout HEK-293T 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. ab126758 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF2 antibody [EPR6048] (ab126758)

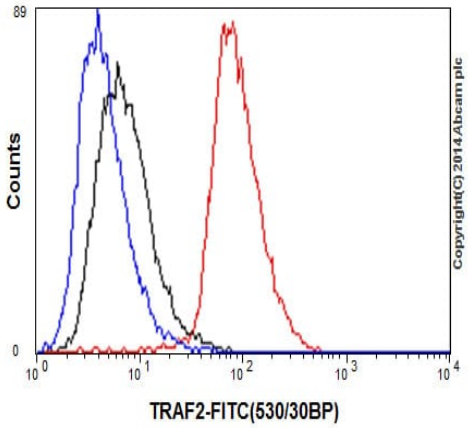
Immunohistochemical staining of paraffin embedded human cervical cancer with purified ab126758 at a working dilution of 1/100. The secondary antibody used is **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-TRAF2 antibody [EPR6048] (ab126758)

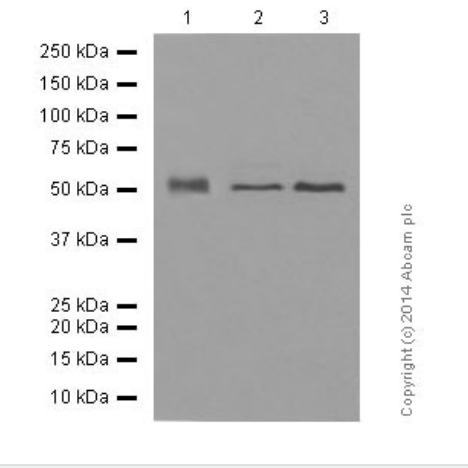
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: TRAF2 knockout HAP1 cell lysate (20 µg)
Lane 3: Human skeletal muscle lysate (20 µg)
Lane 4: U2OS cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab126758 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab126758 was shown to specifically react with TRAF2 when TRAF2 knockout samples were used. Wild-type and TRAF2 knockout samples were subjected to SDS-PAGE. ab126758 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-TRAF2 antibody [EPR6048] (ab126758)

Overlay histogram showing HeLa cells fixed in 2% PFA and stained with purified ab126758 at a dilution of 1 in 120 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control (black) and cells without antibody were used as a negative control (blue).



Western blot - Anti-TRAF2 antibody [EPR6048] (ab126758)

All lanes : Anti-TRAF2 antibody [EPR6048] (ab126758) at 1/2000 dilution (purified)

Lane 1 : Molt-4 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : HEK293 cell lysate

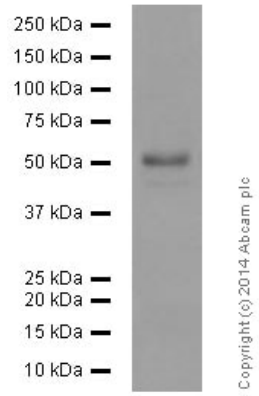
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 55 kDa
Observed band size: 53 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-TRAF2 antibody [EPR6048] (ab126758)

Anti-TRAF2 antibody [EPR6048] (ab126758) at 1/2000 dilution + PC-12 whole cell lysate

Secondary

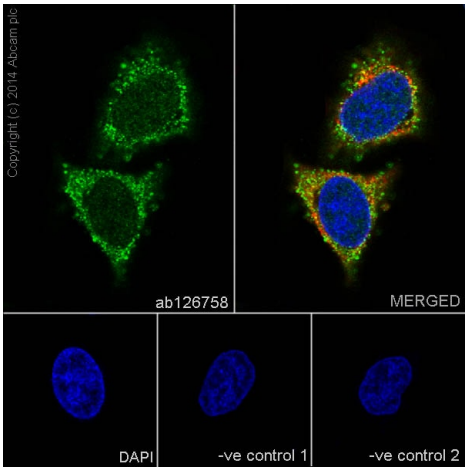
HRP Goat Anti-Rabbit (H+L) at 1/1000 dilution

Predicted band size: 55 kDa

Observed band size: 53 kDa

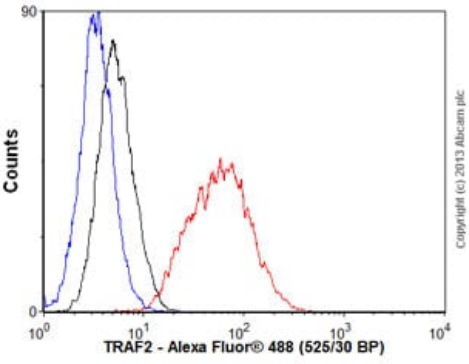
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-TRAF2 antibody [EPR6048] (ab126758)

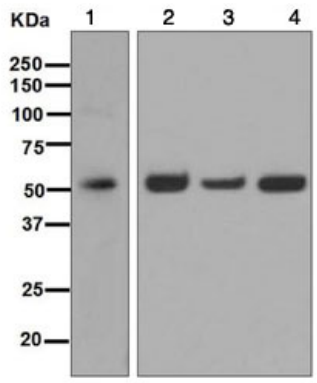
Immunofluorescence staining of HeLa cells with purified ab126758 at a working dilution of 1 in 100, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (**ab7291**) and Alexa Fluor® 594 goat anti-mouse at a dilution of 1/500 (**ab150120**) . The secondary antibody was **ab150077** Alexa Fluor® 488 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in the bottom middle and right hand panels - for the first negative control, purified ab126758 was used at a dilution of 1/200 followed by an Alexa Fluor® 555 goat anti-mouse antibody at a dilution of 1/500 and for the second negative control mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab15007**) were used.



Flow Cytometry (Intracellular) - Anti-TRAF2 antibody [EPR6048] (ab126758)

Overlay histogram showing HeLa cells stained with unpurifiedab126758 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab126758, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW

Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-TRAF2 antibody [EPR6048] (ab126758)

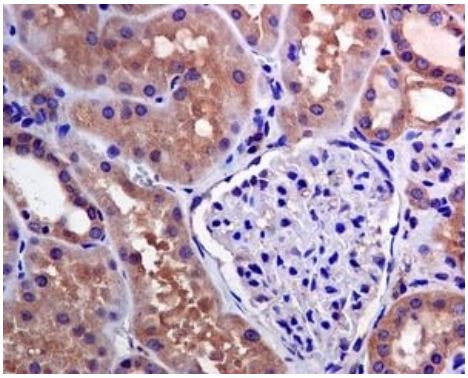
All lanes : Anti-TRAF2 antibody [EPR6048] (ab126758) at 1/1000 dilution (unpurified)

Lane 1 : PC12 cell lysate
Lane 2 : MOLT4 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit at 1/2000 dilution

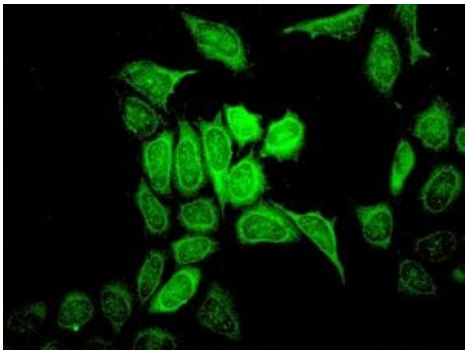
Predicted band size: 55 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TRAF2 antibody [EPR6048] (ab126758)

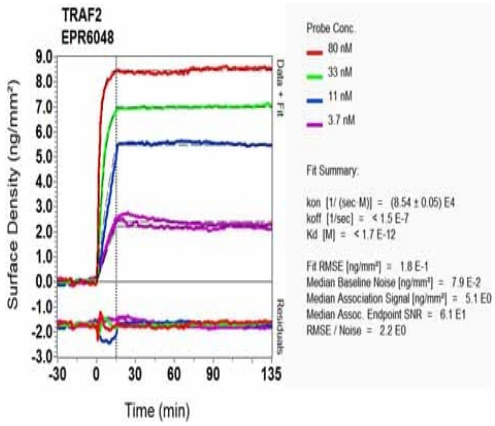
Unpurified ab126758, at 1/50 dilution, staining TRAF2 in paraffin-embedded Human kidney tissue, by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-TRAF2 antibody [EPR6048] (ab126758)

Unpurified ab126758, at 1/100 dilution, staining TRAF2 in HeLa cells, by Immunofluorescence.

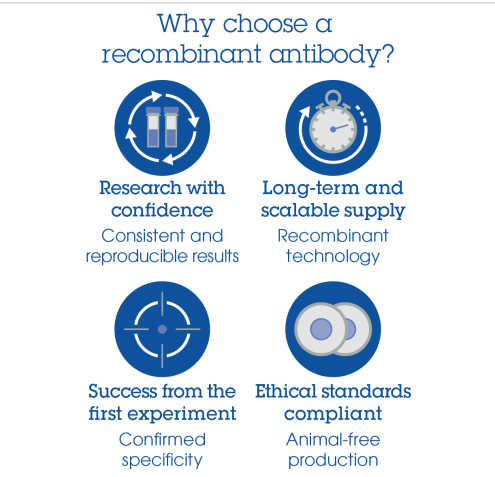


OI-RD Scanning - Anti-TRAF2 antibody [EPR6048] (ab126758)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-TRAF2 antibody [EPR6048] (ab126758)

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