abcam

Product datasheet

Anti-TRAP1 antibody [TRAP1-6] ab2721

14 References 7 Images

Overview

Product name Anti-TRAP1 antibody [TRAP1-6]

Description Mouse monoclonal [TRAP1-6] to TRAP1

Host species Mouse

Specificity Detects tumor necrosis factor receptor-associated protein (TRAP1) from human tissues.

Tested applications Suitable for: IHC-P, IP, WB, ICC/IF

Species reactivity Reacts with: Mouse, Human

Immunogen Recombinant full length protein corresponding to Human TRAP1. Purified recombinant TRAP1.

Database link: Q12931

Positive control ICC/IF: PC-3-M cells

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or

contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 99% PBS

Purity Affinity purified

Primary antibody notes Immunofluorescence staining of TRAP1 in PC-3-M cells with this antibody produces a pattern

consistent with mitochondrial staining. Immunoprecipitation of TRAP1 using this antibody fails to co-precipitate p23, Hop, or CyP40 suggesting TRAP1's inability to associate with these co-

chaperones.

Clonality Monoclonal

1

Clone number

TRAP1-6

Isotype

lgG1

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab2721 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
IHC-P		1/10 - 1/100.
IP		Use at an assay dependent concentration.
WB		1/2000. Detects a band of approximately 76 kDa (predicted molecular weight: 80 kDa).
ICC/IF		1/250.

Target

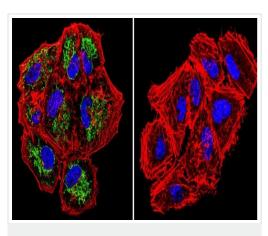
Function Chaperone that expresses an ATPase activity.

Tissue specificity Found in skeletal muscle, liver, heart, brain, kidney, pancreas, lung and placenta.

Sequence similarities Belongs to the heat shock protein 90 family.

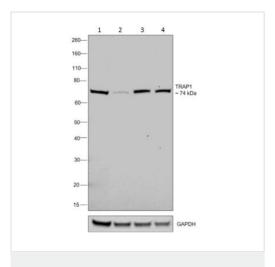
Cellular localization Mitochondrion.

Images



Immunocytochemistry/ Immunofluorescence - Anti-TRAP1 antibody [TRAP1-6] (ab2721)

ab2721 staining TRAP1 in NCI-H460 cells by Immunocytochemistry/Immunofluorescence. Cells were grown on chamber slides and fixed with formaldehyde. Cells were probed without (right) or primary antibody (left) at a dilution of 1:200 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Green - TRAP1, Red (phalloidin) - F-actin, Blue (DAPI) - nuclei. Images were taken at 60X magnification.



Western blot - Anti-TRAP1 antibody [TRAP1-6] (ab2721)

All lanes : Anti-TRAP1 antibody [TRAP1-6] (ab2721) at 1/2000 dilution

Lane 1: HeLa whole cell lysate

Lane 2: Hep G2 whole cell lysate

Lane 3: HEK-293 whole cell lysate

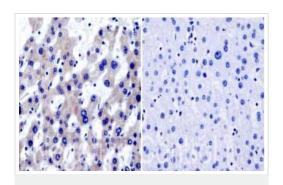
Lane 4: K-562 whole cell lysate

Lysates/proteins at 30 µg per lane.

Secondary

All lanes : Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP at 1/40000 dilution

Predicted band size: 80 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRAP1 antibody [TRAP1-6] (ab2721)

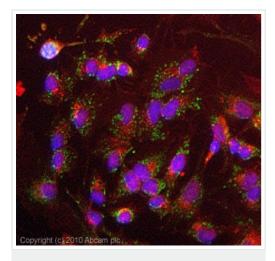
Immunohistochemistry was performed on normal biopsies of deparaffinized Human liver tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer and microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature and probed with a TRAP1 monoclonal antibody (ab2721) at a dilution of 1:20 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

96 kDa 68 kDa 55 kDa 43 kDa

Immunoprecipitation - Anti-TRAP1 antibody [TRAP1-

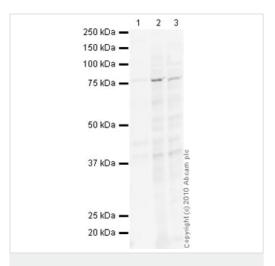
6] (ab2721)

Immunoprecipitation of TRAP1 using ab2721 visualized by Coomassie Blue staining.



Immunocytochemistry/ Immunofluorescence - Anti-TRAP1 antibody [TRAP1-6] (ab2721)

ICC/IF image of ab2721 stained HepG2 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2721, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-TRAP1 antibody [TRAP1-6] (ab2721)

All lanes: Anti-TRAP1 antibody [TRAP1-6] (ab2721) at 1 µg/ml

Lane 1: Human brain tissue lysate - total protein (ab29466)

Lane 2: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 3: HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat polyclonal Secondary Antibody to Mouse IgG -H&L (HRP), pre-adsorbed at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

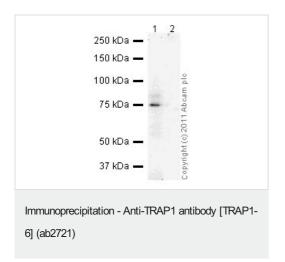
Predicted band size: 80 kDa **Observed band size:** 76 kDa

Additional bands at: 40 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 16 minutes

The band observed at 76 kDa could potentially be a cleaved form of TRAP1 due to the presence of a 59 amino acid transit peptide.



TRAP1 was immunoprecipitated using 0.5mg HepG2 whole cell extract, 5μg of Mouse monoclonal to TRAP1 (ab2721)and 50μl of protein G magnetic beads (lane 1). The antibody was incubated with the Protein G beads for 10min under agitation. No antibody was added to the control (lane 2). HepG2 whole cell extract diluted in RIPA buffer was added to each sample and incubated for 10min under agitation. Proteins were eluted by addition of 40μl SDS loading buffer and incubated for 10min at 70°C; 10μl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab2721. Secondary: Goat polyclonal to mouse lgG light chain specific (HRP) at 1/5000 dilution.

Bands: 75kDa: TRAP1

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