abcam

Product datasheet

Anti-RanGAP1 antibody [EPR3295] ab92360

Recombinant RabMAb

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Overview

Product name	Anti-RanGAP1 antibody [EPR3295]
Description	Rabbit monoclonal [EPR3295] to RanGAP1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P, Flow Cyt, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. within Human RanGAP1 aa 1-100. The exact sequence is proprietary. Database link: P46060
Positive control	WB: MCF7 (Human breast adenocarcinoma cell line) Cytoplasmic Lysate - tumor cell line (ab29538), HeLa, SH SY5Y and A549 cell lysates, mouse and rat brain tissue lysates. IHC-P: Human breast carcinoma, Human testis, Human bladder carcinoma, Mouse testis and Rat liver tissue. ICC/IF: mouse hepatocyte, and MCF7 cells. FC: Jurkat cells IP: HeLa cell lysate
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

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.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Purification notes	This antibody is not purified. It is provided in cell supernatant & storage buffer

Clonality	Monoclonal
Clone number	EPR3295
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab92360** in the following tested applications.

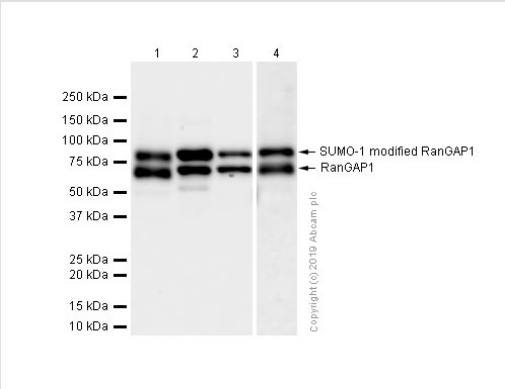
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	1/1000 - 1/5000. Predicted molecular weight: 64 kDa.
IP		1/20. **For unpurified use at 1/10**
IHC-P		1/500. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. **For unpurified use at 1/100 - 1/250.** See IHC antigen retrieval protocols.
Flow Cyt	★★★★★	1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. **For unpurified use at 1/50**
ICC/IF	★★★★★	1/100 - 1/250.

Target

Function	GTPase activator for the nuclear Ras-related regulatory protein Ran, converting it to the putatively inactive GDP-bound state.
Tissue specificity	Highly expressed in brain, thymus and testis.
Sequence similarities	Belongs to the RNA1 family. Contains 6 LRR (leucine-rich) repeats.
Post-translational modifications	Phosphorylated occurs before nuclear envelope breakdown and continues throughout mitosis. Phosphorylated by the M-phase kinase cyclin B/Cdk1, in vitro. Differential timimg of dephosphorylation occurs during phases of mitosis. The phosphorylated form remains associated with RANBP2/NUP358 and the SUMO E2-conjugating enzyme, UBC9, on nuclear pore complex (NPC) diassembly and during mitosis. Sumoylated with SUMO1. Sumoylation is necessary for targeting to the nuclear envelope (NE), and for association with mitotic spindles and kinetochores during mitosis. Also required for interaction with RANBP2 and is mediated by UBC9.
Cellular localization	Cytoplasm. Nucleus membrane. Chromosome, centromere, kinetochore. Cytoplasm, cytoskeleton, spindle pole. Cytoplasmic during interphase. Targeted to the nuclear rim after sumoylation. During mitosis, associates with mitotic spindles. Association with kinetochores appears soon after nuclear envelope breakdown and persists until late anaphase. Mitotic location also requires sumoylation.

Images



Western blot - Anti-RanGAP1 antibody [EPR3295] (ab92360)

All lanes : Anti-RanGAP1 antibody [EPR3295] (ab92360) at 1/1000 dilution (Purified)

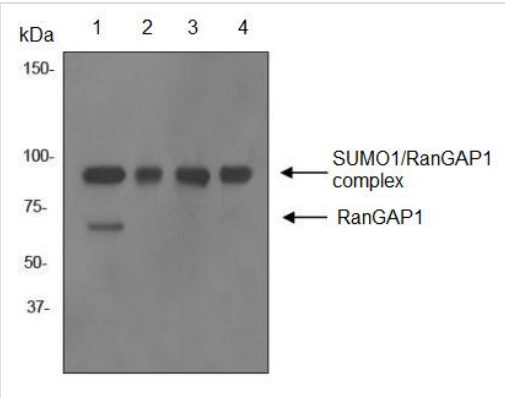
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysates
Lane 3 : Mouse brain lysates
Lane 4 : Rat brain lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 64 kDa
Observed band size: 70,90 kDa
why is the actual band size different from the predicted?

The doublets detected are consistent with what have been described in literature PMID: 24988324 and PMID: 21646468



Western blot - Anti-RanGAP1 antibody [EPR3295] (ab92360)

All lanes : Anti-RanGAP1 antibody [EPR3295] (ab92360) at 1/1000 dilution

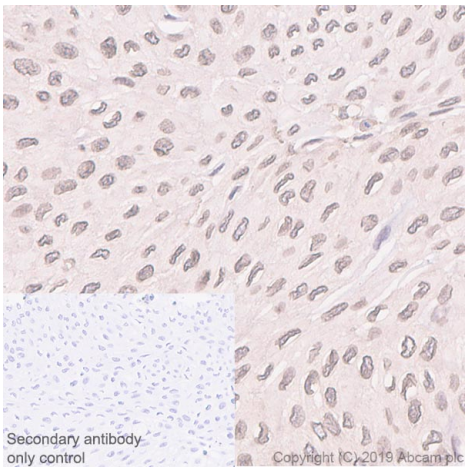
Lane 1 : HeLa cell lysate
Lane 2 : MCF-7 cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 10 µg per lane.

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

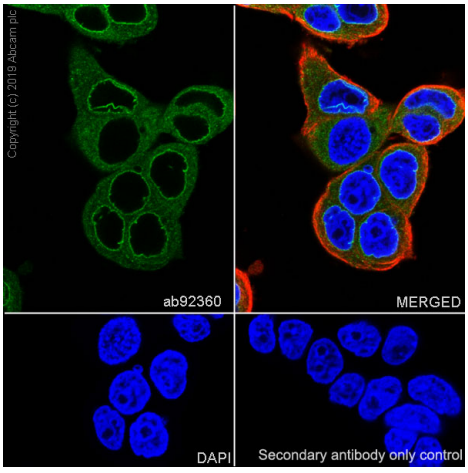
Predicted band size: 64 kDa
Observed band size: 64 kDa

Additional bands at: 90 kDa. We are unsure as to the identity of these extra bands.



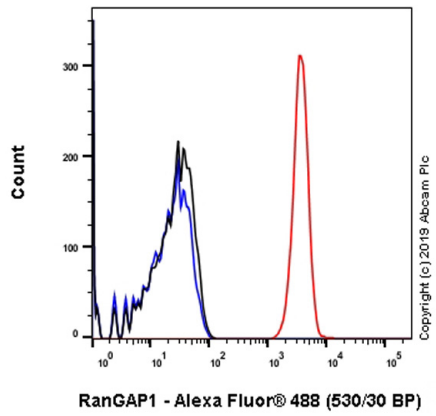
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human bladder carcinoma tissue sections labeling RanGAP1 with purified ab92360 at 1/500 dilution (0.22 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



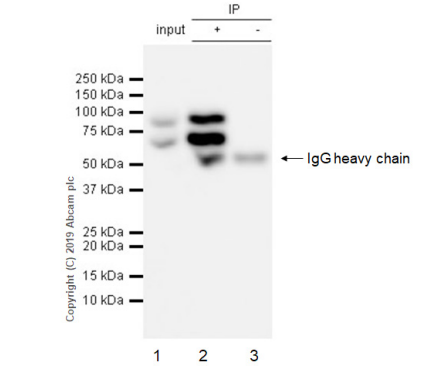
Immunocytochemistry/ Immunofluorescence - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling RanGAP1 with purified ab92360 at 1/100 dilution (1.1 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml) dilution. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



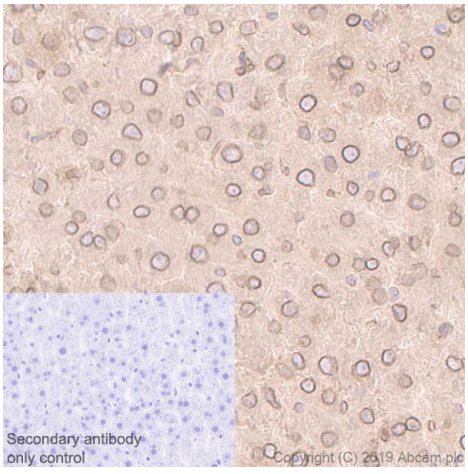
Flow Cytometry - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling RanGAP1 with purified ab92360 at 1/20 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



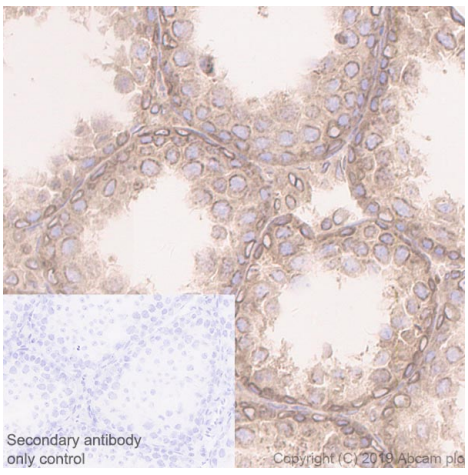
Immunoprecipitation - Anti-RanGAP1 antibody [EPR3295] (ab92360)

ab92360 (purified) at 1/20 dilution (0.5ug) immunoprecipitating RanGAP1 in HeLa whole cell lysates.
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates 10ug
Lane 2 (+): ab92360 & HeLa whole cell lysates
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab92360 in HeLa whole cell lysates
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



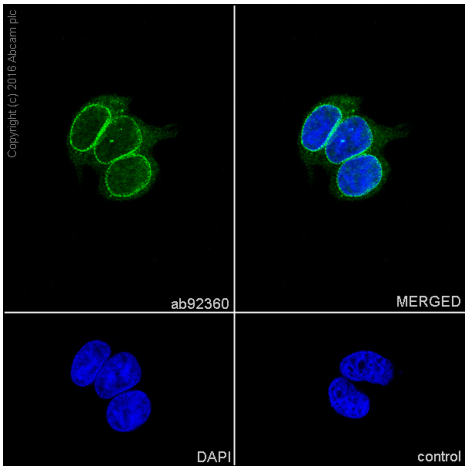
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling RanGAP1 with purified ab92360 at 1/500 dilution (0.22 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



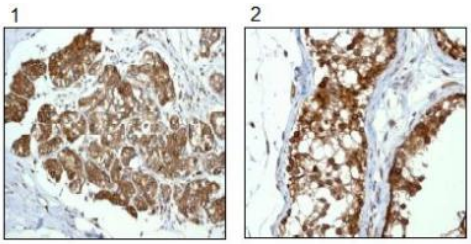
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue sections labeling RanGAP1 with purified ab92360 at 1/500 dilution (0.22 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



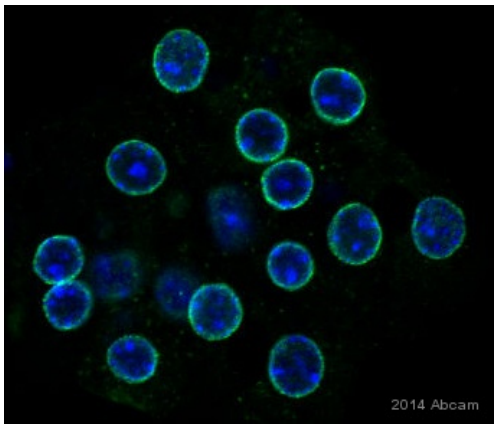
Immunocytochemistry/ Immunofluorescence - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Immunofluorescence staining of MCF7 cells with purified ab92360 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RanGAP1 antibody [EPR3295] (ab92360)

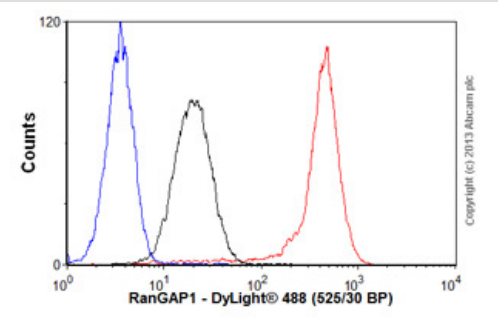
b92360 at 1/100 dilution staining RanGAP1 in paraffin-embedded (1) Human breast carcinoma tissue and (2) Human testis tissue by immunohistochemistry.



Immunocytochemistry/ Immunofluorescence - Anti-RanGAP1 antibody [EPR3295] (ab92360)

This image is courtesy of an anonymous Abreview

ab92360 staining RanGAP1 in mouse hepatocyte cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized, and blocked with 2% BSA for 2 hours at 22°C. Samples were incubated with primary antibody (1/100 in blocking buffer) for 18 hours at 4°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/10000) was used as the secondary antibody.



Flow Cytometry - Anti-RanGAP1 antibody [EPR3295] (ab92360)

Overlay histogram showing Jurkat cells stained with ab92360 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab92360, 1/100 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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