abcam

ab32373

Anti-RAP1GAP antibody [Y134]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y134
Purification technique	Affinity purification Protein A
Specificity	ab32373 recognises Rap1 GTPase-activating protein 1
Concentration	0.166 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Rat
Dilution info	1/20 - 1/60
Notes	-

Expected

Species	Mouse, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/10000 - 1/50000
Notes	-

Species	Rat
Dilution info	1/10000 - 1/50000
Notes	-

Species	Human
Dilution info	1/10000 - 1/50000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/30 - 1/50
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information RAP1GAP

Function	GTPase activator for the nuclear Ras-related regulatory protein RAP-1A (KREV-1), converting it to the putatively inactive GDP-bound state.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

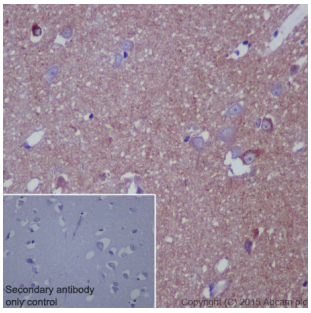
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

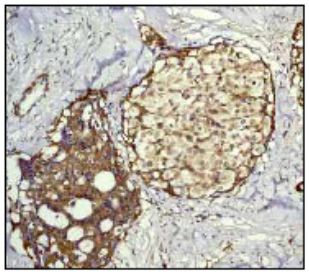
12 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-RAP1GAP antibody

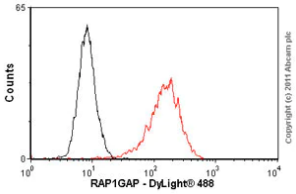
Immunohistochemical staining of paraffin embedded human cerebral cortex with purified ab32373 at a working dilution of 1/1000. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-RAP1GAP antibody

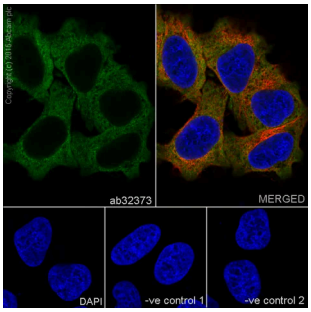
Immunohistochemical staining of paraffin-embedded human breast cancer tissue using unpurified ab32373 at 1/100 dilution.



Flow Cytometry (Intracellular) - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Flow Cytometry (Intracellular) staining using rabbit Anti-RAP1GAP antibody

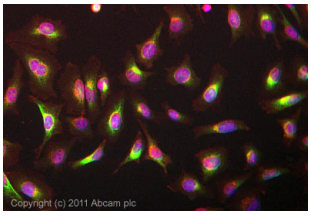
Overlay histogram showing SH-SY5Y cells stained with unpurified ab32373 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32373 1/100) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 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. Acquisition of >5000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RAP1GAP antibody

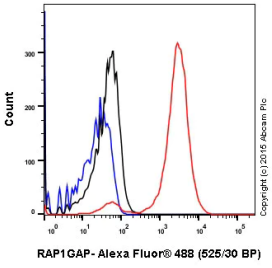
Immunofluorescence staining of HeLa cells with purified ab32373 at a working dilution of 1/250 counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077) used at a dilution of 1/1000. ab7291 a mouse anti-tubulin antibody (1/1000) was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse 1/1000) shown in the top right hand panel. The cells were fixed in 4 % PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1 purified ab32373 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2 ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



Immunocytochemistry/ Immunofluorescence - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RAP1GAP antibody

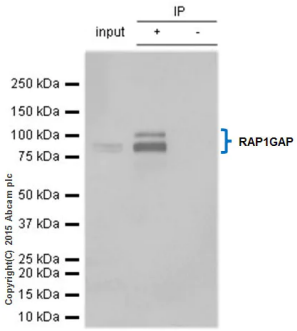
ICC/IF image of unpurified ab32373 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32373 1µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899 DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 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.



Flow Cytometry (Intracellular) - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Flow Cytometry (Intracellular) staining using rabbit Anti-RAP1GAP antibody

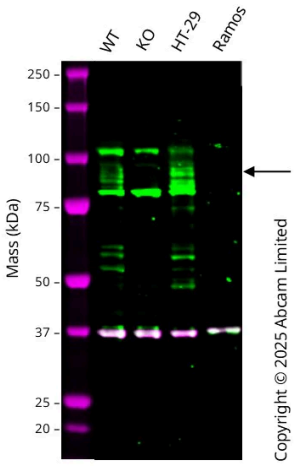
Overlay histogram showing Jurkat cells fixed in 4% PFA and stained with purified ab32373 at a dilution of 1/30 (red line). The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit at a dilution of 1/500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Immunoprecipitation - Anti-RAP1GAP antibody [Y134] (ab32373)

ab32373 (purified) at 1/20 immunoprecipitating RAP1GAP in 10 µg C6 cell lysate (Lanes 1 and 2, observed at 82-95 kDa). Lane 3 - Rabbit monoclonal IgG (ab172730). For western blotting, HRP Veriblot for IP Detection Reagent (ab131366) was used for detection (1/1000). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST
All lanes:
Immunoprecipitation - Anti-RAP1GAP antibody [Y134] (ab32373)

Predicted band size: 73 kDa



Western blot - Anti-RAP1GAP antibody [Y134] (ab32373)

RAP1GAP Western blot staining using rabbit Anti-RAP1GAP antibody

Western blot: Anti-RAP1GAP antibody [Y134] ab32373 staining at 1/10000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 90 kDa in Wild-type A549 cell lysates with no signal observed at this size in RAP1GAP knockout A549 cell line.

To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.
Lanes 1 - 4:
Western blot - Anti-RAP1GAP antibody [Y134] (ab32373) at 1/10000 dilution

Lanes 1 - 4:
Western blot - Anti-RAP1GAP antibody [Y134] - BSA and Azide free (ab247250) at 1/10000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
RAP1GAP knockout A549 at 20 µg

Lane 3:
HT-29 at 20 µg

Lane 4:
Ramos at 20 µg

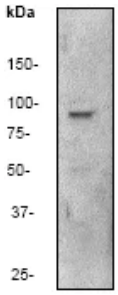
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 73 kDa

Observed band size: 90 kDa



Western blot - Anti-RAP1GAP antibody [Y134] (ab32373)

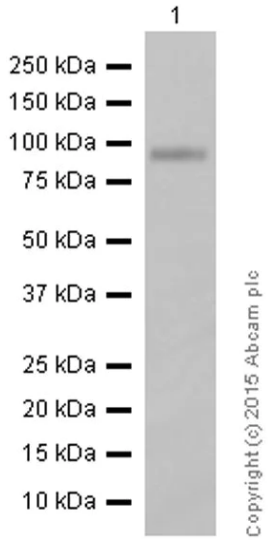
The molecular weight observed is consistent with what has been described in the literature (PMID: 9346962, PMID: 15632203, PMID: 30144784).

All lanes:
Western blot - Anti-RAP1GAP antibody [Y134] (ab32373) at 1/50000 dilution

All lanes:
Jurkat cell lysate

Predicted band size: 73 kDa

Observed band size: 95 kDa



Western blot - Anti-RAP1GAP antibody [Y134] (ab32373)

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The molecular weight observed is consistent with what has been described in the literature (PMID: 9346962, PMID: 15632203, PMID: 30144784).

All lanes:
Western blot - Anti-RAP1GAP antibody [Y134] (ab32373) at 1/10000 dilution

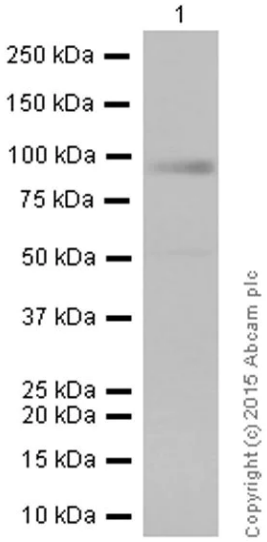
All lanes:
SH-SY5Y cell lysate at 20 µg

Secondary

All lanes:
Anti-rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 73 kDa

Observed band size: 95 kDa



Western blot - Anti-RAP1GAP antibody [Y134] (ab32373)

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The molecular weight observed is consistent with what has been described in the literature (PMID: 9346962, PMID: 15632203, PMID: 30144784).

All lanes:
Western blot - Anti-RAP1GAP antibody [Y134] (ab32373) at 1/10000 dilution

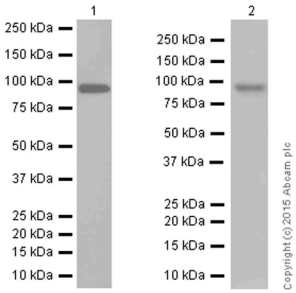
All lanes:
Jurkat cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 73 kDa

Observed band size: 95 kDa



Western blot - Anti-RAP1GAP antibody [Y134] (ab32373)

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The molecular weight observed is consistent with what has been described in the literature (PMID: 9346962, PMID: 15632203, PMID: 30144784).

All lanes:
Western blot - Anti-RAP1GAP antibody [Y134] (ab32373) at 1/10000 dilution

Lane 1:
Mouse brain lysate at 20 µg

Lane 2:
Rat brain lysate at 20 µg

Secondary

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

Predicted band size: 73 kDa

Observed band size: 95 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.