abcam

ab201687

Anti-GEF H1 antibody [EPR17963] - C-terminal

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	EPR17963
Purification technique	Affinity purification Protein A
Concentration	0.594 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	-

Species	Human

Dilution info	1/2000
Notes	-

Species	Rat
Dilution info	1/2000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse
Dilution info	1/500
Notes	-

Species	Rat
Dilution info	1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse

Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Rat
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IP

Tested

Species	Human
Dilution info	2 µg
Notes	-

Expected

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

Target data

See full target information ARHGEF2

Function

Activates Rho-GTPases by promoting the exchange of GDP for GTP. May be involved in epithelial barrier permeability, cell motility and polarization, dendritic spine morphology, antigen presentation, leukemic cell differentiation, cell cycle regulation, innate immune response, and cancer. Binds Rac-GTPases, but does not seem to promote nucleotide exchange activity toward Rac-GTPases, which was uniquely reported in PubMed:9857026. May stimulate instead the cortical activity of Rac. Inactive toward CDC42, TC10, or Ras-GTPases. Forms an intracellular sensing system along with NOD1 for the detection of microbial effectors during cell invasion by pathogens. Required for RHOA and RIP2 dependent NF-kappaB signaling pathways activation upon S.flexneri cell invasion. Involved not only in sensing peptidoglycan (PGN)-derived muropeptides through NOD1 that is independent of its GEF activity, but also in the activation of NF-kappaB by Shigella effector proteins (IpgB2 and OspB) which requires its GEF activity and the activation of RhoA. Involved in innate immune signaling transduction pathway promoting cytokine IL6/interleukin-6 and TNF secretion in macrophage upon

stimulation by bacterial peptidoglycans; acts as a signaling intermediate between NOD2 receptor and RIPK2 kinase. Contributes to the tyrosine phosphorylation of RIPK2 through Src tyrosine kinase leading to NF-kappaB activation by NOD2. Overexpression activates Rho-, but not Rac-GTPases, and increases paracellular permeability (By similarity). Involved in neuronal progenitor cell division and differentiation (PubMed:28453519). Involved in the migration of precerebellar neurons (By similarity).

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.

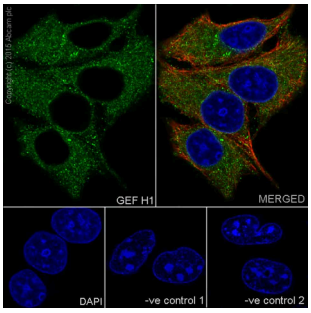
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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10 product images



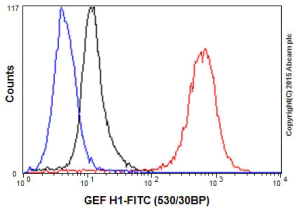
Immunocytochemistry/ Immunofluorescence - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GEF H1 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling GEF H1 with ab201687 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).
Confocal image showing cytoplasmic staining on HeLa cell line is observed.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows;
1. ab201687 at 1/500 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

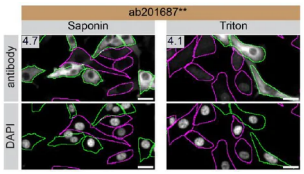


Flow Cytometry (Intracellular) - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Flow Cytometry (Intracellular) staining using rabbit Anti-GEF H1 antibody

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed HEK293 (human embryonic kidney) cells labeling GEF H1 with ab201687 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and a unlabelled control (cells without incubation with

primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.

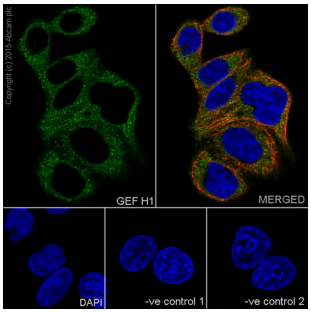


Immunocytochemistry/ Immunofluorescence - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GEF H1 antibody

ab201687 was shown to react with ARHGEF2 in wild-type U-87 MG cells in immunocytochemistry with loss of signal observed in a ARHGEF2 siRNA knockdown cell line. Wild-type and siRNA knockdown cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab201687 at 1/500 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (siRNA knockdown) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and siRNA knockdown cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss (LSM-880).

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



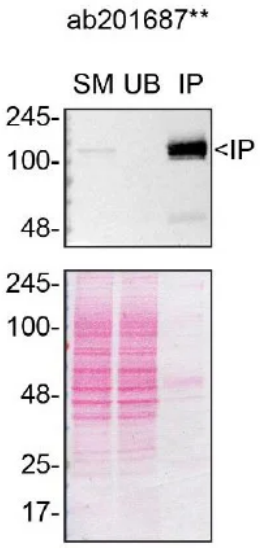
Immunocytochemistry/ Immunofluorescence - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GEF H1 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (Human breast adenocarcinoma cell line) cells labeling GEF H1 with ab201687 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).
Confocal image showing cytoplasmic staining on MCF7 cell line is observed.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows;
1. ab201687 at 1/500 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



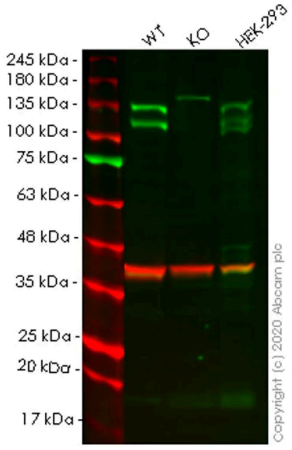
Immunoprecipitation - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

Immunoprecipitation of ARHGEF2 in U-87 MG cells. Lysates were prepared and immunoprecipitation was performed using 2 µg of ab201687 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Immunoprecipitation - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 2 µg

All lanes:
U-87 MG cell lysates

Observed band size: 111.5 kDa



Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Western blot staining using rabbit Anti-GEF H1 antibody

Lanes 1-3: Merged signal (red and green). Green - ab201687 observed at 75 kDa. Red - loading control, ab8245 observed at 37 kDa.
ab201687 Anti-GEF H1 antibody [EPR17963] - C-terminal was shown to specifically react with GEF H1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265797 (knockout cell lysate ab257223) was used. Wild-type and GEF H1 knockout samples were subjected to SDS-PAGE. ab201687 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

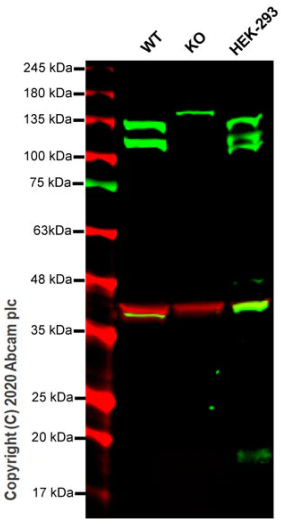
Lane 2:
ARHGEF2 knockout HeLa cell lysate at 20 µg

Lane 3:
HEK293 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 111 kDa

Observed band size: 75 kDa



Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Western blot staining using rabbit Anti-GEF H1 antibody

Lanes 1-3: Merged signal (red and green). Green - ab201687 observed at 112 kDa. Red - loading control, ab8245 observed at 37 kDa.
ab201687 Anti-GEF H1 antibody [EPR17963] - C-terminal was shown to specifically react with GEF H1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265797 (knockout cell lysate ab257223) was used. Wild-type and GEF H1 knockout samples were subjected to SDS-PAGE. ab201687 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
ARHGEF knockout HeLa cell lysate at 20 µg

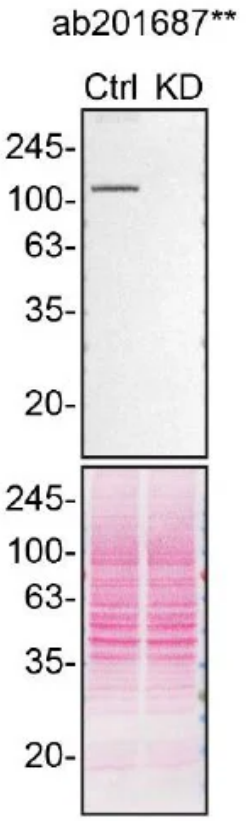
Lane 2:
Western blot - Human ARHGEF2 (GEF H1) knockout HeLa cell line (ab265797)

Lane 3:
HEK-293T cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 111 kDa

Observed band size: 112 kDa, 75 kDa



Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

GEF H1 Western blot staining using rabbit Anti-GEF H1 antibody

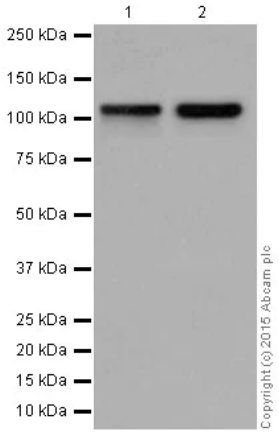
ab201687 was shown to react with ARHGEF2 in wild-type U-87 MG cells in Western blot with loss of signal observed in a ARHGEF2 siRNA knockdown cell line. Cell lysates from wild-type U-87 MG transfected with either scrambled siRNA or ARHGEF2 siRNA were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab201687 overnight at 4 °C at a 1/2000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 1/2000 dilution

Lane 1:
Wild-type U-87 MG transfected with scrambled siRNA control lysate at 30 µg

Lane 2:
U-87 MG transfected with siRNA specifically targeting ARHGEF2 cell lysate at 30 µg

Observed band size: 111.5 kDa



Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 1/10000 dilution

Lane 1:
HEK293 (Human embryonic kidney) lysate at 20 µg

Lane 2:
HEK293 (Human epithelial cells from cervix adenocarcinoma) lysate at 20 µg

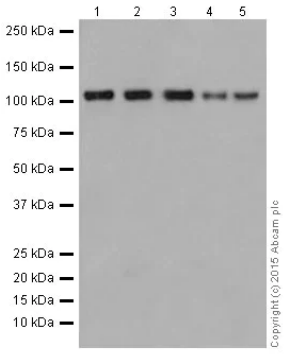
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 111 kDa

Observed band size: 112 kDa

Exposure time: 30s



Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-GEF H1 antibody [EPR17963] - C-terminal (ab201687) at 1/2000 dilution

Lane 1:
Mouse brain lysate at 10 µg

Lane 2:
Rat brain lysate at 10 µg

Lane 3:
Raw264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) lysate at 10 µg

Lane 4:
C6 (Rat glial tumor cells) lysate at 10 µg

Lane 5:
NIH/3T3 (Mouse embyro fibroblast cells) at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 111 kDa

Observed band size: 112 kDa

Exposure time: 15s

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