abcam

ab18056

Anti-HEF1/NEDD-9 antibody [2G9]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.2 Preservative: 0.1% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Human NEDD9 aa 50-400. The exact immunogen used to generate this antibody is proprietary information. Database link Q14511
Clone number	2G9
Purification technique	Affinity purification Protein G
Specificity	Not tested on Sin1. This antibody mostly detects HEF1 / NEDD-9 localized at the focal adhesion sites.
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC

Tested

Species	Human

Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

IP

Expected

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

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.

Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	-
Notes	-

Species	Human
Dilution info	0.1 µg/mL
Notes	-

Expected

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

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse

Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Expected

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

IHC-Fr

Expected

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

Target data

See full target information NEDD9

Function

Scaffolding protein which plays a central coordinating role for tyrosine-kinase-based signaling related to cell adhesion (PubMed:24574519). As a focal adhesion protein, plays a role in embryonic fibroblast migration (By similarity). May play an important role in integrin beta-1 or B cell antigen receptor (BCR) mediated signaling in B- and T-cells. Integrin beta-1 stimulation leads to recruitment of various proteins including CRKL and SHPTP2 to the tyrosine phosphorylated form (PubMed:9020138). Promotes adhesion and migration of lymphocytes; as a result required for the correct migration of lymphocytes to the spleen and other secondary lymphoid organs (PubMed:17174122). Plays a role in the organization of T-cell F-actin cortical cytoskeleton and the centralization of T-cell receptor microclusters at the immunological synapse (By similarity). Negatively regulates cilia outgrowth in polarized cysts (By similarity). Modulates cilia disassembly via

activation of AURKA-mediated phosphorylation of HDAC6 and subsequent deacetylation of alpha-tubulin (PubMed:17604723). Positively regulates RANKL-induced osteoclastogenesis (By similarity). Required for the maintenance of hippocampal dendritic spines in the dentate gyrus and CA1 regions, thereby involved in spatial learning and memory (By similarity).

Storage

Shipped at conditions	Blue Ice
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

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	HEF1 also known as NEDD9 is an intracellular signaling protein and a member of the Crk-associated substrate family. It has a molecular mass of approximately 93 kDa. The expression of this protein occurs mainly in tissues involved in cell movement and proliferation like lymphoid organs and neural tissues. It is part of the CAS (Crk-associated substrate) proteins hence associated with a wide range of cellular mechanics.
Biological function summary	HEF1 functions as a scaffolding protein involved in various cellular processes including cell adhesion migration and proliferation. It interacts with several other proteins and forms part of complexes such as focal adhesion complexes. It regulates the dynamics of these complexes by modulating signaling pathways linked to cellular adhesion and migration. In neuronal cells it influences axonal and dendritic branching.
Pathways	HEF1 plays a significant role in both the integrin signaling pathway and cell cycle regulation. Through these pathways HEF1 interacts with various key proteins including focal adhesion kinase (FAK) and Src kinase. It positions itself strategically within these pathways and facilitates cross-talk between signaling molecules enabling the coordination of structural and replicative functions in cells.
Associated diseases and disorders	HEF1 has been implicated in the progression of cancers such as breast cancer and melanoma. Its overexpression often correlates with increased metastatic potential.

In breast cancer HEF1 interacts with proteins like Aurora A and BRCA1 linking it to pathways involved in tumor proliferation and invasion. In melanoma alterations in HEF1 levels can affect melanoma cell migration making it a potential target for therapeutic intervention.

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

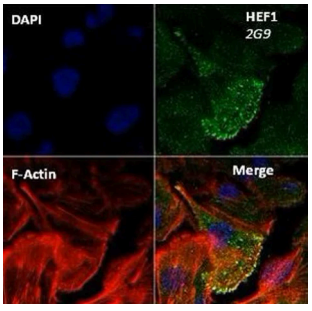
We do not recommend this combination. It is not covered by our product promise.

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.

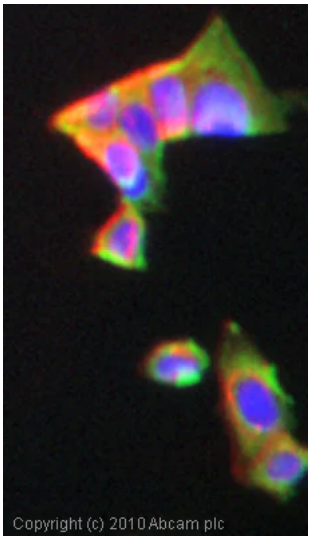
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8 product images



Immunocytochemistry/ Immunofluorescence - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

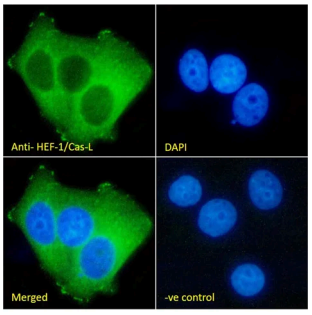
Immunofluorescence staining of MDA-MB231 cells with the 2G9 antibody at a 1/200 dilution. Cells were fixed in 3.8% PFA for 10 minutes, and staining was performed for 1 hour at room temperature.



Immunocytochemistry - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

HEF1/NEDD-9 Immunocytochemistry staining using mouse Anti-HEF1/NEDD-9 antibody

ICC/IF image of ab18056 stained MCF7 (human breast adenocarcinoma cell line) cells. The cells were fixed in 4% formaldehyde for 10 minutes and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab18056, 1 µg/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 1 hour. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.

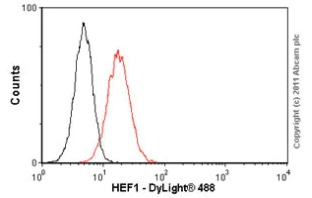


Immunocytochemistry/ Immunofluorescence - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

Immunofluorescence analysis of paraformaldehyde fixed MCF7 cells, permeabilized with 0.15% Triton.

Primary incubation 1hr (1:100 dilution) followed by Alexa Fluor® 488 secondary antibody (1:1000 dilution), showing cytoplasmic staining. The nuclear stain is DAPI (blue).

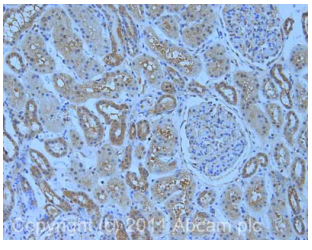
Negative control: Mouse IgG1 negative control followed by Alexa Fluor® 488 secondary antibody.



Flow Cytometry - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

HEF1/NEDD-9 Flow Cytometry staining using mouse Anti-HEF1/NEDD-9 antibody

Overlay histogram showing A549 (human lung carcinoma cell line) cells stained with ab18056 (red line). The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. 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 (ab18056, 1 µg/ 1 x 10^6 cells) for 30 minutes at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2 µg/ 1 x 10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in A549 cells fixed with 4% paraformaldehyde (10 minutes)/permeabilized in 0.1% PBS-Tween used under the same conditions.

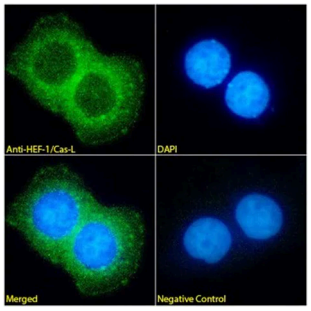


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

HEF1/NEDD-9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-HEF1/NEDD-9 antibody

IHC image of ab18056 staining in human kidney formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab18056, 10 µg/ml, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

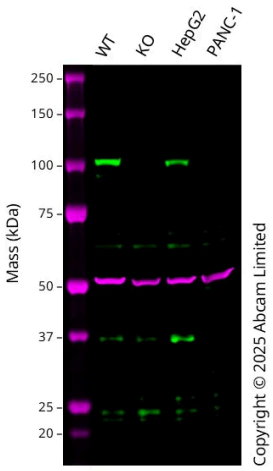


Immunocytochemistry/ Immunofluorescence - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

Immunofluorescence analysis of paraformaldehyde fixed MCF7 cells, permeabilized with 0.15% Triton.

Primary incubation 1hr (1:100 dilution) followed by Alexa Fluor® 488 secondary antibody (1:1000 dilution), showing cytoplasmic staining. The nuclear stain is DAPI (blue).

Negative control: Mouse IgG1 negative control followed by Alexa Fluor® 488 secondary antibody.



Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

HEF1/NEDD-9 Western blot staining using mouse Anti-HEF1/NEDD-9 antibody

Western blot: Anti-HEF1/NEDD-9 antibody [2G9] ab18056 staining at 0.1 µg/mL, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 100 kDa in Wild-type A549 cell lysates with no signal observed at this size in NEDD9 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-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056) at 0.1 µg/mL

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
NEDD9 knockout A549 at 20 µg

Lane 3:
HepG2 at 20 µg

Lane 4:
PANC-1 at 20 µg

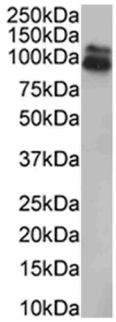
Secondary

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

Performed under reducing conditions.

Predicted band size: 93 kDa

Observed band size: 100 kDa



Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

(0.1µg/ml) staining in MCF7 cells lysate (35µg protein in RIPA buffer). Primary incubation was 1 hour. Detected by chemiluminescence.
All lanes:
Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056) at 0.1 µg/mL

Predicted band size: 92 kDa

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