

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

★★★★★ [9 Abreviews](#) [30 References](#) [画像数 7](#)

製品の概要

製品名	Anti-HEF1/NEDD-9 antibody [2G9]
製品の詳細	Mouse monoclonal [2G9] to HEF1/NEDD-9
由来種	Mouse
特異性	Not tested on Sin1. This antibody mostly detects HEF1 / NEDD-9 localized at the focal adhesion sites.
アプリケーション	適用あり: IP, ICC/IF, IHC-Fr, ICC, IHC-P, WB, Flow Cyt
種交差性	交差種: Mouse, Rat, Human
免疫原	Fusion protein corresponding to Human HEF1/NEDD-9 aa 50-400. Database link: Q14511
ポジティブ・コントロール	WB: Whole cell lysate prepared from a murine neural stem cell line. ICC/IF: MCF7 cells. Flow Cytometry: A549 cells. IHC-P: Human kidney tissue.
特記事項	<p>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.</p> <p>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</p>

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.20 Preservative: 0.1% Sodium azide Constituent: PBS
精製度	Protein G purified
ポリ/モノ	モノクローナル
クローン名	2G9

ミエローマ	Sp2/0
アイソタイプ	IgG1
軽鎖の種類	kappa

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab18056の使用に適用されます**
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IP	★★★★★ (1)	Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration. PubMed: 19376971
IHC-Fr		Use at an assay dependent concentration. PubMed: 19464348
ICC		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
WB	★★★★★ (4)	Use at an assay dependent concentration.
Flow Cyt		Use 1µg for 10 ⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

ターゲット情報

機能	Docking protein which plays a central coordinating role for tyrosine-kinase-based signaling related to cell adhesion. May function in transmitting growth control signals between focal adhesions at the cell periphery and the mitotic spindle in response to adhesion or growth factor signals initiating cell proliferation. 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 CRK, NCK and SHPTP2 to the tyrosine phosphorylated form.
組織特異性	Widely expressed. Higher levels detected in kidney, lung, and placenta. Also detected in T-cells, B-cells and diverse cell lines. The protein has been detected in lymphocytes, in diverse cell lines, and in lung tissues.
配列類似性	Belongs to the CAS family. Contains 1 SH3 domain.
ドメイン	Contains a central domain containing multiple potential SH2-binding sites and a C-terminal domain containing a divergent helix-loop-helix (HLH) motif. The SH2-binding sites putatively bind CRK, NCK and ABL SH2 domains. The HLH motif confers specific interaction with the HLH proteins ID2, E12 and E47. It is absolutely required for the induction of pseudohyphal growth in yeast and mediates homodimerization and heterodimerization with p130cas. The SH3 domain interacts with two proline-rich regions of focal adhesion kinase.

翻訳後修飾

Cell cycle-regulated processing produces four isoforms: p115, p105, p65, and p55. Isoform p115 arises from p105 phosphorylation and appears later in the cell cycle. Isoform p55 arises from p105 as a result of cleavage at a caspase cleavage-related site and it appears specifically at mitosis. The p65 isoform is poorly detected.

Focal adhesion kinase 1 phosphorylates the protein at the YDYVHL motif (conserved among all cas proteins). The SRC family kinases (FYN, SRC, LCK and CRK) are recruited to the phosphorylated sites and can phosphorylate other tyrosine residues. Ligation of either integrin beta-1 or B-cell antigen receptor on tonsillar B-cells and B-cell lines promotes tyrosine phosphorylation and both integrin and BCR-mediated tyrosine phosphorylation requires an intact actin network. In fibroblasts transformation with oncogene v-ABL results in an increase in tyrosine phosphorylation. Transiently phosphorylated following CD3 cross-linking and this phosphorylated form binds to CRK and C3G. A mutant lacking the SH3 domain is phosphorylated upon CD3 cross-linking but not upon integrin beta-1 cross-linking. Tyrosine phosphorylation occurs upon stimulation of the G-protein coupled C1a calcitonin receptor in rabbit. Calcitonin-stimulated tyrosine phosphorylation is mediated by calcium- and protein kinase C-dependent mechanisms and requires the integrity of the actin cytoskeleton.

細胞内局在

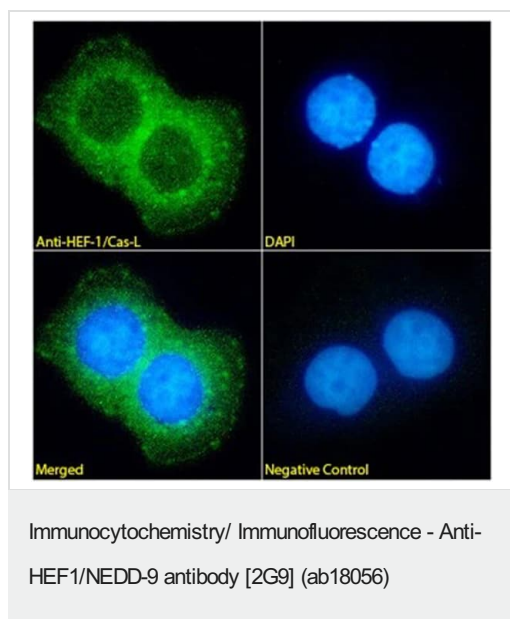
Cytoplasm > cytoskeleton > spindle and Cytoplasm > cell cortex. Nucleus. Golgi apparatus. Cell projection > lamellipodium. Cytoplasm. Cell junction > focal adhesion. Localizes to both the cell nucleus and the cell periphery and is differently localized in fibroblasts and epithelial cells. In fibroblasts is predominantly nuclear and in some cells is present in the Golgi apparatus. In epithelial cells localized predominantly in the cell periphery with particular concentration in lamellipodia but is also found in the nucleus. Isoforms p105 and p115 are predominantly cytoplasmic and associate with focal adhesions while p55 associates with mitotic spindle.

画像

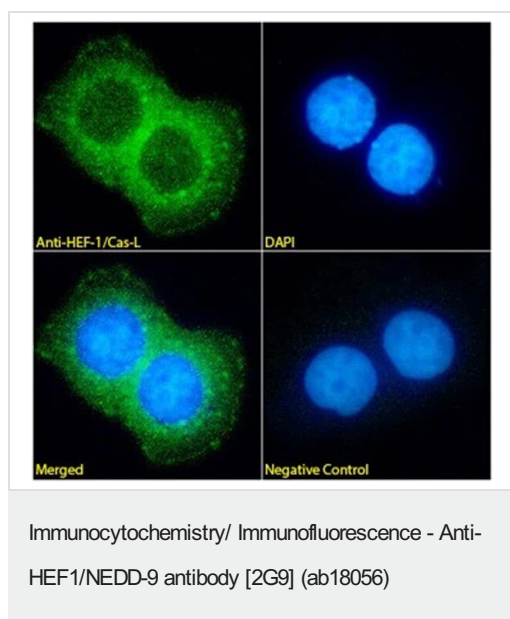


(0.1µg/ml) staining in MCF7 cells lysate (35µg protein in RIPA buffer). Primary incubation was 1 hour. Detected by chemiluminescence.

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



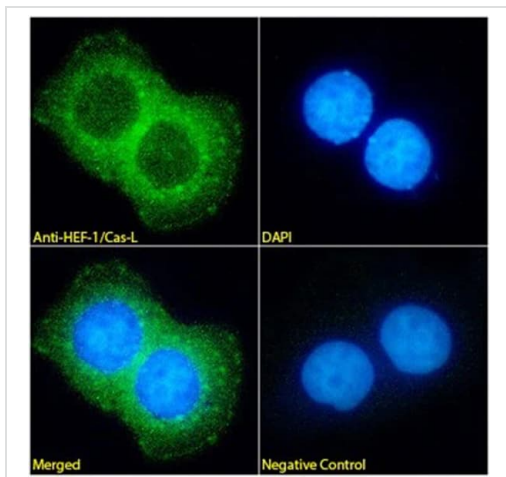
Immunofluorescence staining of MDA-MB231 cells with the 2G9 antibody at a 1/200 dilution. Cells were fixed in 3.8% PFA for 10 minutes, and staining was performed for 1 hour at room temperature.



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.

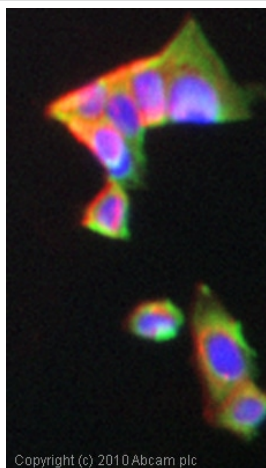


Immunocytochemistry/ Immunofluorescence - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

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

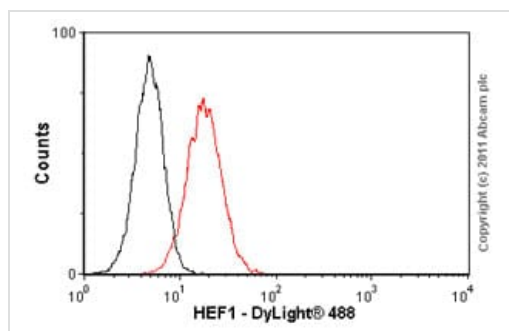
Primary incubation 1hr (1:100 dilution) followed by Alexa Fluor® 488 secondary antibody (1:1000 dilution), showing cytoplasmic staining. The nuclear stain is DAPI (blue).

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



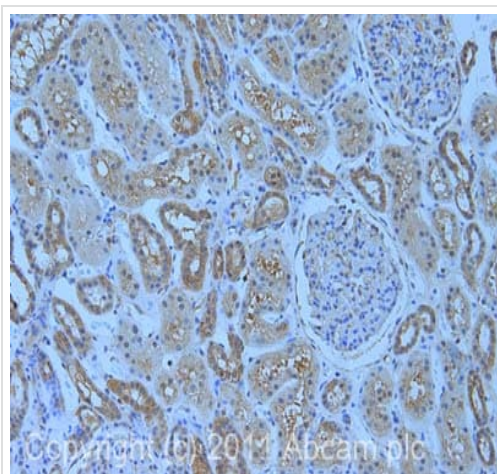
Immunocytochemistry - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

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.



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

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 10⁶ 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 10⁶ 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)

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.

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