abcam

Product datasheet

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

★★★★★ 9 Abreviews 30 References 7 图像

概述

产品名称	Anti-HEF1/NEDD-9抗体[2G9]
描述	**小鼠单克隆抗体**[2G9] to HEF1/NEDD-9
宿主	Mouse
特异性	Not tested on Sin1. This antibody mostly detects HEF1 / NEDD-9 localized at the focal adhesion sites.
经测试应用	**适用于:** IP, ICC/IF, IHC-Fr, ICC, IHC-P, WB, Flow Cyt
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Fusion protein corresponding to Human HEF1/NEDD-9 aa 50-400. Database link: **Q14511**
阳性对照	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.
常规说明	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. 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

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.20 Preservative: 0.1% Sodium azide Constituent: PBS
纯度	Protein G purified
克隆	单克隆
克隆编号	2G9

骨髓瘤	Sp2/0
同种型	IgG1
轻链类型	kappa

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab18056于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

功能 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.

组织特异性 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.

序列相似性 Belongs to the CAS family.
Contains 1 SH3 domain.

结构域 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.

翻译后修饰

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.

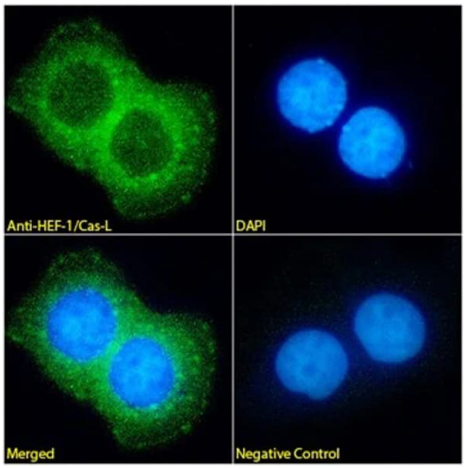
细胞定位

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.

图片

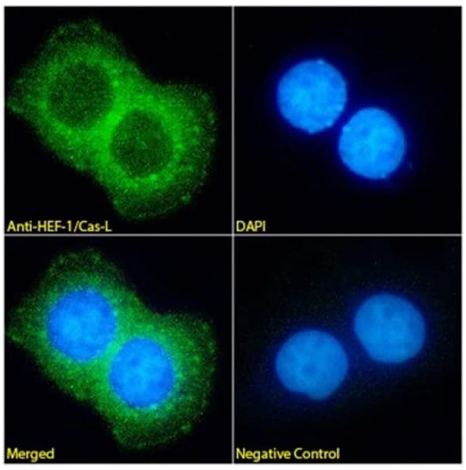
Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

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



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.

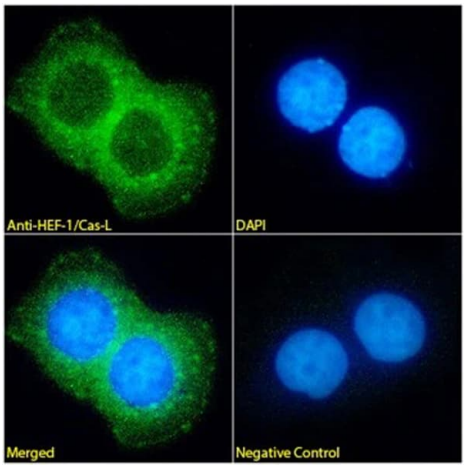


Immunocytochemistry/ Immunofluorescence - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

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.



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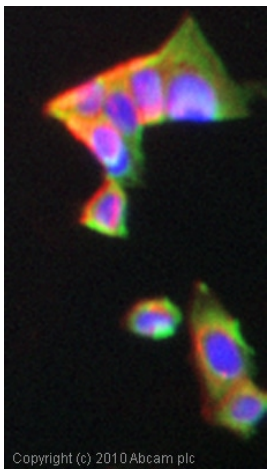
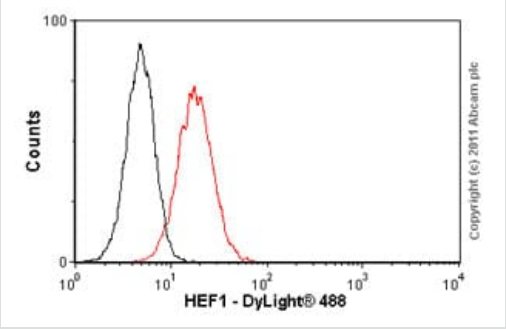


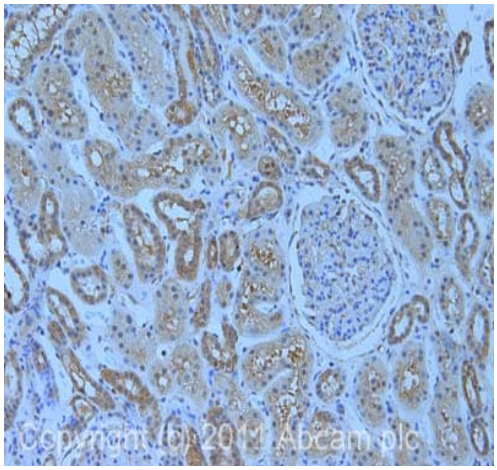
Immunocytochemistry - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056)

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 10^6 cells) for 30 minutes at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 minutes at 22ºC. 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)

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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