Datasheet for ABIN129518

anti-NEDD9 antibody (AA 82-398)

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Overview

Quantity:	100 µg
Target:	NEDD9
Binding Specificity:	AA 82-398
Reactivity:	Human, Rat, Mouse
Host:	Mouse
Clonality:	Monoclonal
Application:	Western Blotting (WB), ELISA, Immunofluorescence (IF), Immunoprecipitation (IP), Fluorescence Microscopy (FM)

Product Details

Purpose:	HEF1 Antibody
Immunogen:	Immunogen: Anti-HEF1 monoclonal antibody was produced by repeated immunizations with a synthetic peptide corresponding to amino acid residues 82-398 of human HEF1 protein (hHEF1, 843 aa, predicted MW 92.8 kDa). Immunogen Type: Conjugated Peptide
Clone:	2G9
Isotype:	IgG1 kappa
Cross-Reactivity (Details):	This Protein A purified antibody is directed against human HEF1 protein.
Characteristics:	Synonyms: mouse anti-hEF1 antibody, mouse anti-NEDD-9 antibody, mouse anti-CASL antibody, Cas like docking antibody, CASL antibody, Crk associated substrate related protein antibody, dJ49G10.2 antibody, dJ761I2.1 antibody, Enhancer of filamentation 1 antibody

Product Details

Purification:	The product was purified from tissue culture supernatant by chromatography.
Sterility:	Sterile filtered

Target Details

Target:	NEDD9
Alternative Name:	NEDD9 (NEDD9 Products)
Background:	Background: HEF1, also known as Enhancer of filamentation 1, CRK-associated substrate-related protein, CAS-L, CasL, p105 and Neural precursor cell expressed developmentally down-regulated 9 is the product of the NEDD9 (CASGL) gene. HEF1 functions as a docking protein that plays a central coordinating role for tyrosine-kinase-based signaling related to cell adhesion. HEF1 may also 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. HEF1 may also 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. HEF1 forms a homodimer and can heterodimerize with HLH proteins ID2, E12, E47 and also with p130cas. HEF1 also forms complexes in vivo with related adhesion focal tyrosine kinase (RAFTK), adapter protein CRKL and LYN kinase and also interacts with MICAL and TXNL4/DIM1. This protein localizes to both the cell nucleus and the cell periphery and is differently localized in fibroblasts and epithelial cells. In fibroblasts, it is predominantly nuclear and in some cells is present in the Golgi apparatus. In epithelial cells, it is localized predominantly in the cell periphery with particular concentration in lamellipodia, but it is also found in the nucleus. HEF1 is widely expressed although higher levels are detected in kidney, lung, and placental tissue. HEF1 is also detected in T-cells, B-cells and diverse cell lines. HEF1 is activated upon induction of cell growth. 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. Isoforms p105 and p115 are predominantly cytoplasmic and associate with focal adhesions while p55 associates with the mitotic spindle.
Gene ID:	4739, 5453680
UniProt:	Q14511

Application Details

Application Notes:	Application Note: This monoclonal antibody has been tested for use in western blotting, immunoprecipitation and immunofluorescence. Specific conditions for reactivity should be optimized by the end user. Expect bands approximately 115 and 105 in size corresponding to isoforms of HEF1 protein by western blotting in the appropriate cell lysate or extract. This antibody does not recognize p130Cas. Sin1 has not been tested. IF was performed using 4 % PFA fixed cells. This monoclonal antibody mostly detects HEF1 localized at the focal adhesion sites. Western Blot Dilution: 1:5,000 Immunoprecipitation Dilution: 1:1,000 ELISA Dilution: 1:5,000 - 1:20,000 IF Microscopy Dilution: 1:500 Other: User Optimized
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Buffer:	Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: None Preservative: 0.01 % (w/v) Sodium Azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiry Date:	12 months

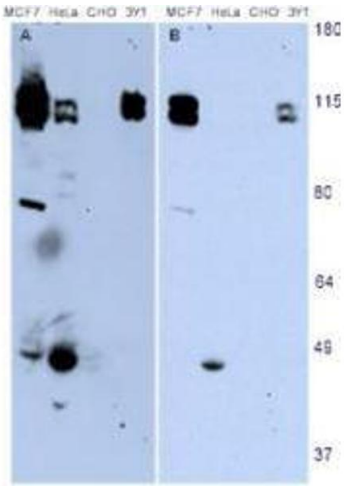
Publications

Product cited in:	Pugacheva, Golemis: "The focal adhesion scaffolding protein HEF1 regulates activation of the

Publications

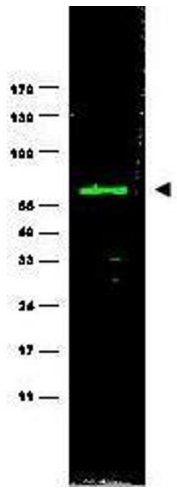
Aurora-A and Nek2 kinases at the centrosome." in: **Nature cell biology**, Vol. 7, Issue 10, pp. 937-46, (2005) (PubMed).

Images



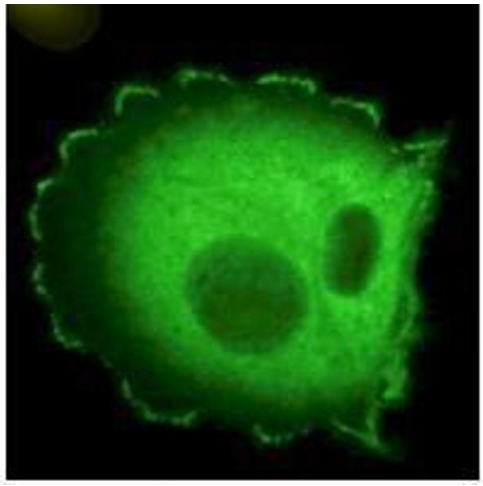
Western Blotting

Image 1. Western blotting using monoclonal anti-HEF1 antibody (clone 2G9) shows detection of endogenous HEF1 present in various cell lines. Panel A shows detection using a 15 min exposure. Panel B is the same blot exposed for 2 min. The doublet represents p105 and p115 staining. The lower MW band represents p55. 3Y1 cells are derived from rat fibroblast cells. Mouse 3T3 cells are also reactive (not shown). To date no staining has been noted on CHO cells. Personal Communication. Elena Pugacheva, Fox Chase Cancer Center, Philadelphia, PA.



Western Blotting

Image 2. Western blot using monoclonal anti-HEF1 antibody (clone 2G9) antibody shows detection of a 115 kDa band corresponding to HEF1 in MCF7 lysate (arrowhead). Approximately 35 ?g of lysate was loaded for SDS-PAGE followed by transfer onto nitrocellulose and reaction with a 1:1,000 dilution of anti-HEF1 antibody. Detection occurred using a 1:5,000 dilution of800 conjugated Sh-a-Mouse IgG [H&L] for 45 min at room temperature (800 nm channel, green). Molecular weight estimation was made by comparison to prestained MW markers (indicated at left).800 fluorescence image was captured using the Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



Immunofluorescence

Image 3. Immunofluorescence microscopy using Monoclonal anti-HEF1 antibody (clone 2G9) shows detection of HEF1 localized at focal adhesion sites. The antibody was used at a 1:500 dilution with a 3-sec exposure time. Personal Communication. Elena Pugacheva, Fox Chase Cancer Center, Philadelphia, PA.