BOSTER BIOLOGICAL TECHNOLOGY
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Anti-HEF1 NEDD9 Monoclonal Antibody

Catalog Number: M00838

About NEDD9

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.

Overview

Product Name	Anti-HEF1 NEDD9 Monoclonal Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-HEF1 NEDD9 Monoclonal Antibody (Catalog # M00838). Tested in IF, IP, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, IP, IF, WB
Clonality	Monoclonal Clone: 2G9
Formulation	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01% (w/v) Sodium Azide
Storage Instructions	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. Expiration date is one (1) year from date of opening. (Ship on dry ice.)
Host	Mouse
Uniprot ID	Q14511

Technical Details

BOSTER
antibody and ELISA experts

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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).
Predicted Reactive Species	Canine
Isotype	IgG1 kappa
Form	Liquid (sterile filtered)
Concentration	1.0 mg/mL by UV absorbance at 280 nm
Purification	This Protein A purified antibody is directed against human HEF1 protein. The product was purified from tissue culture supernatant by chromatography. Reactivity occurs against human, mouse and rat forms of the protein. Reactivity against multiple isoforms is expected. Reactivity against homologues from other sources is not known. Specificity was determined by partial epitope mapping.
Suggested Dilutions	ELISA: 1:5,000 - 1:20,000 IF Microscopy: 1:500 IP: 1:1,000 WB: 1:5,000 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.

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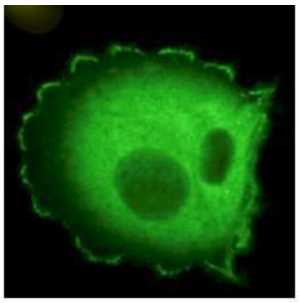
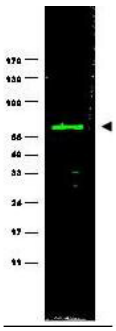
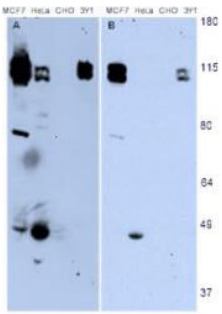
Anti-HEF1 NEDD9 Monoclonal Antibody (M00838) Images



Immunofluorescence microscopy using Boster's 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.



Western blot using Boster's monoclonal anti-HEF1 antibody (clone 2G9) antibody shows detection of a ~92 kDa band corresponding to HEF1 in MCF7 lysate [arrowhead]. Approximately 35 µg 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 of IRDye®800 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). IRDye®800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



Western blotting using Boster's monoclonal anti-HEF1 antibody (clone 2G9) shows detection of endogenous HEF1 present in various cell lines [MCF7, HeLa, CHO, 3Y1]. 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.