SANTA CRUZ BIOTECHNOLOGY, INC.

RLF (D-7): sc-365373

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

c-Jun is an important transcription factor that is involved in the regulation of proliferation, differentiation and cellular transformation induced by oncogenic Ras. An activated Ras effector, RLF (also designated as Ras-associated protein Rab2L/RalGDS-like factor), a guanine nucleotide exchange factor (GEF) of the small GTPase Ral, induces the phosphorylation of Serines 63 and 73 of c-Jun. The RalGEF-Ral pathway plays an important role in Ras-dependent c-Jun phosphoryation. RLF functions as an intermediate between Ras and Ral pathways by binding to the GTP-bound form of Ras proteins through its C-terminal Ras-binding domain (RBD), which is very similar to that of RalGDS-RBD. RLF-induced Ral activation is stimulated by Ras. RLF, when targeted to the plasma membrane using the Ras farnesyl attachment site (RLF-CAAX), is constitutively active to induce both Ral activation and c-Fos promoter activity. RLF mediates a distinct Ras-induced signaling pathway to gene induction and RLF-CAAX stimulates both trancriptional activation and cell growth. Overexpression of RLF-CAAX induces neuroretina cell division, but has no effect on ERK activity, whereas inhibition of MEK blocks both Ras- and RLF-CAAX-induced differentiation, suggesting that RalGEFs induce differentiation depending on the basal MEK or ERK activity.

CHROMOSOMAL LOCATION

Genetic locus: RGL2 (human) mapping to 6p21.32; Rgl2 (mouse) mapping to 17 B1.

SOURCE

RLF (D-7) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 7-33 near the N-terminus of RLF of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

RLF (D-7) is available conjugated to agarose (sc-365373 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-365373 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-365373 PE), fluorescein (sc-365373 FITC), Alexa Fluor® 488 (sc-365373 AF488), Alexa Fluor® 546 (sc-365373 AF546), Alexa Fluor® 594 (sc-365373 AF594) or Alexa Fluor® 647 (sc-365373 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-365373 AF680) or Alexa Fluor® 790 (sc-365373 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

Blocking peptide available for competition studies, sc-365373 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

Alexa Fluor® is a trademark of Molecular Probes, Inc., Oregon, USA

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

APPLICATIONS

RLF (D-7) is recommended for detection of RLF of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

RLF (D-7) is also recommended for detection of RLF in additional species, including equine, bovine and porcine.

Suitable for use as control antibody for RLF siRNA (h): sc-41846, RLF siRNA (m): sc-41847, RLF shRNA Plasmid (h): sc-41846-SH, RLF shRNA Plasmid (m): sc-41847-SH, RLF shRNA (h) Lentiviral Particles: sc-41846-V and RLF shRNA (m) Lentiviral Particles: sc-41847-V.

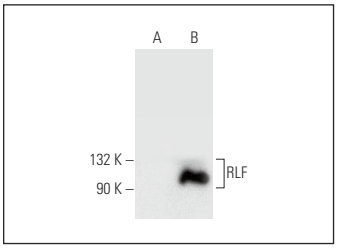
Molecular Weight of RLF: 84 kDa.

Positive Controls: RLF (h): 293T Lysate: sc-115319 or HeLa whole cell lysate: sc-2200.

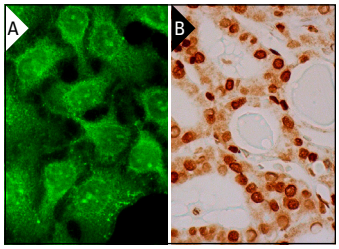
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850. 4) Immunohistochemistry: use m-IgGκ BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

DATA



RLF (D-7): sc-365373. Western blot analysis of RLF expression in non-transfected: sc-117752 (**A**) and human RLF transfected: sc-115319 (**B**) 293T whole cell lysates.



RLF (D-7): sc-365373. Immunofluorescence staining of methanol-fixed HeLa cells showing nuclear and cytoplasmic localization (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human thyroid gland tissue showing nuclear and cytoplasmic staining of glandular cells (**B**).

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.