

RLF (h): 293T Lysate: sc-115319

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 phosphorylation. 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 transcriptional 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.

REFERENCES

1. Wolthuis, R.M., et al. 1997. Stimulation of gene induction and cell growth by the Ras effector RLF. *EMBO J.* 16: 6738-6761.
2. Esser, D., et al. 1998. Structure determination of the Ras-binding domain of the Ral-specific guanine nucleotide exchange factor RLF. *Biochemistry* 37: 13453-13462.
3. Wolthuis, R.M., et al. 1998. Ras-dependent activation of the small GTPase Ral. *Curr. Biol.* 8: 471-474.
4. Verheijen, M.H., et al. 1999. Interdependent action of RalGEF and Erk in Ras-induced primitive endoderm differentiation of F9 embryonal carcinoma cells. *Oncogene* 18: 4435-4439.
5. de Ruiter, N.D., et al. 2000. Ras-dependent regulation of c-Jun phosphorylation is mediated by the Ral guanine nucleotide exchange factor-Ral pathway. *Mol. Cell. Biol.* 20: 8480-8488.
6. Sood, R., et al. 2000. The human RGL (RalGDS-like) gene: cloning, expression analysis and genomic organization. *Biochim. Biophys. Acta* 1491: 285-288.

CHROMOSOMAL LOCATION

Genetic locus: RGL2 (human) mapping to 6p21.32.

PRODUCT

RLF (h): 293T Lysate represents a lysate of human RLF transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

APPLICATIONS

RLF (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive RLF antibodies. Recommended use: 10-20 µl per lane.

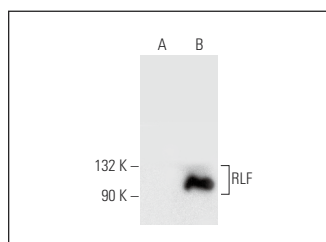
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

RLF (D-7): sc-365373 is recommended as a positive control antibody for Western Blot analysis of enhanced human RLF expression in RLF transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

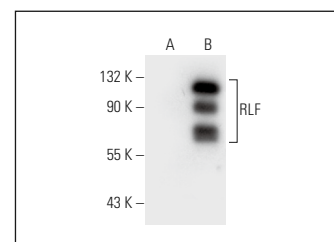
RECOMMENDED SUPPORT REAGENTS

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.

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 (E-4): sc-393670. Western blot analysis of RLF expression in non-transfected: sc-117752 (A) and human RLF transfected: sc-115319 (B) 293T whole cell lysates.

RESEARCH USE

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

PROTOCOLS

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