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SANTA CRUZ BIOTECHNOLOGY, INC.

RLF siRNA (h): sc-41846

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. Over-expression 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. Stiumlation 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. Peyssonnaux, C., et al. 2000. Induction of postmitotic neuroretina cell proliferation by distinct Ras downstream signaling pathways. Mol. Cell. Biol. 20: 7068-7079.

CHROMOSOMAL LOCATION

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

PRODUCT

RLF siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RLF shRNA Plasmid (h): sc-41846-SH and RLF shRNA (h) Lentiviral Particles: sc-41846-V as alternate gene silencing products.

For independent verification of RLF (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41846A, sc-41846B and sc-41846C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RLF siRNA (h) is recommended for the inhibition of RLF expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

RLF (D-7): sc-365373 is recommended as a control antibody for monitoring of RLF gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RLF gene expression knockdown using RT-PCR Primer: RLF (h)-PR: sc-41846-PR (20 µl, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.