



GRB2 siRNA (m): sc-29335

BACKGROUND

The superfamily of GTP binding proteins, of which Ras proteins are prototypes, has been implicated in a broad range of biological activities. A family of guanine nucleotide releasing factors (GRFs) activate Ras in mammalian cells and growth factor receptor-bound protein 2 (GRB2), an adaptor protein (also referred to as Sem 5) that appears to mediate the interaction of GRFs with activated receptor molecules. GRB2 forms a complex with activated EGFR (epidermal growth factor receptor) and the Ras-specific guanine nucleotide exchange factor Sos1, and, together, they regulate the growth factor-induced activation of Ras. GRB2 exhibits both structural and functional homology to the *C. elegans* protein Sem-5. GRB2 is necessary during embryogenesis for the differentiation of endodermal cells and formation of the epiblast.

REFERENCES

1. Lowenstein, E.J., et al. 1992. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to Ras signaling. *Cell* 40: 431-442.
2. Chardin, P., et al. 1993. Human Sos 1: a guanine nucleotide exchange factor for Ras that binds to GRB2. *Science* 260: 1338-1343.
3. Skolnik, E.Y., et al. 1993. The function of GRB2 in linking the Insulin receptor to Ras signaling pathways. *Science* 260: 1953-1955.
4. Simon, M.A., et al. 1993. An SH3-SH2-SH3 protein is required for p21 Ras 1 activation and binds to sevenless and Sos proteins *in vitro*. *Cell* 73: 169-177.
5. Buday, L. and Downward, J. 1993. Epidermal growth factor regulates p21 Ras through the formation of a complex of receptor, GRB2 adaptor protein, and Sos nucleotide exchange factor. *Cell* 73: 611-620.
6. Egan, S.E., et al. 1993. Association of Sos Ras exchange protein with GRB2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 363: 45-51.
7. Zhang, X., et al. 1993. Normal and oncogenic p21 Ras proteins bind to the amino-terminal regulatory domain of c-RAF-1. *Nature* 364: 308-313.

CHROMOSOMAL LOCATION

Genetic locus: Grb2 (mouse) mapping to 11 E2.

PRODUCT

GRB2 siRNA (m) is a target-specific 19-25 nt siRNA designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GRB2 shRNA Plasmid (m): sc-29335-SH and GRB2 shRNA (m) Lentiviral Particles: sc-29335-V as alternate gene silencing products.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GRB2 siRNA (m) is recommended for the inhibition of GRB2 expression in mouse 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 μ M in 66 μ l. 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

GRB2 (C-7): sc-8034 is recommended as a control antibody for monitoring of GRB2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 GRB2 gene expression knockdown using RT-PCR Primer: GRB2 (m)-PR: sc-29335-PR (20 μ l, 497 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Breitenbuecher, F., et al. 2009. A novel molecular mechanism of primary resistance to Flt3-kinase inhibitors in AML. *Blood* 113: 4063-4073.
2. Ahmed, Z., et al. 2013. Grb2 controls phosphorylation of FGFR2 by inhibiting receptor kinase and Shp2 phosphatase activity. *J. Cell Biol.* 200: 493-504.

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.