

GRB7 siRNA (h): sc-35510

BACKGROUND

Many growth factors function by binding receptors with intrinsic tyrosine kinase activity. Signaling by such receptors involves a series of intermediates characterized by SH2 domains that bind tyrosine phosphorylated receptors by a direct interaction between the SH2 domain and the phosphotyrosine-containing receptor sequences. GRB7, a SH2 domain protein, has a single SH2 domain at its C-terminal, a central region with similarity to Ras GAP and a proline-rich N-terminus. GRB7 maps to the region on mouse chromosome 11 containing the Neu gene. This region of mouse chromosome 11 is syntenic to an area of human chromosome 17q that is frequently amplified in breast cancer. Moreover, GRB7 is amplified and over-expressed in breast cancer and is found in a complex with Neu gp185.

REFERENCES

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2. Schlessinger, J., et al. 1992. Growth factor signalling by receptor tyrosine kinases. *Neuron* 9: 383-391.
3. Margolis, B. 1992. Proteins with SH2 domains: transducers in the tyrosine kinase signalling pathway. *Cell Growth Differ.* 3: 73-80.
4. Margolis, B., et al. 1992. High-efficiency expression/cloning of epidermal growth factor-receptor-binding proteins with Src homology 2 domains. *Proc. Natl. Acad. Sci. USA* 89: 8894-8898.
5. Fantl, W.J., et al. 1993. Signalling by receptor tyrosine kinases. *Annu. Rev. Biochem.* 62: 453-481.
6. Pawson, T., et al. 1993. SH2 and SH3 domains. *Curr. Biol.* 3: 434-442.
7. Stein, D., et al. 1994. The SH2 domain protein GRB7 is co-amplified, overexpressed and in a tight complex with HER2 in breast cancer. *EMBO J.* 13: 1331-1340.

CHROMOSOMAL LOCATION

Genetic locus: GRB7 (human) mapping to 17q12.

PRODUCT

GRB7 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GRB7 shRNA Plasmid (h): sc-35510-SH and GRB7 shRNA (h) Lentiviral Particles: sc-35510-V as alternate gene silencing products.

For independent verification of GRB7 (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-35510A, sc-35510B and sc-35510C.

PROTOCOLS

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

GRB7 siRNA (h) is recommended for the inhibition of GRB7 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 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

GRB7 (A-12): sc-376069 is recommended as a control antibody for monitoring of GRB7 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 GRB7 gene expression knockdown using RT-PCR Primer: GRB7 (h)-PR: sc-35510-PR (20 μ l, 527 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.