

Gads siRNA (h): sc-40958

BACKGROUND

The Src homology 3 (SH3) region is a small protein domain of approximately 60 amino acids present in a large group of proteins. In general, it exists in association with catalytic domains, as in the nonreceptor protein-tyrosine kinases and phospholipase C- γ , within structural proteins, such as spectrin or Myosin, and in small adapter proteins, such as Crk and GRB2. SH3 domains are often accompanied by SH2 domains of 100 amino acids that bind to tyrosine-phosphorylated regions of target proteins, frequently linking activated growth factors to putative signal transduction proteins. Deletion or mutation of SH3 domains generally activates the transforming potential of nonreceptor tyrosine kinases, suggesting that SH3 mediates negative regulation of an intrinsic transforming activity. Gads is an adapter proteins that contains both SH2 and SH3 domains. Gads binds to tyrosine-phosphorylated proteins, such as Shc, and functions to couple these proteins to downstream effectors.

REFERENCES

1. Ullrich, A. and Schlessinger, J. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell* 61: 203-212.
2. Ellis, C., et al. 1990. Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. *Nature* 343: 377-381.
3. Morrison, D.K., et al. 1990. Platelet-derived growth factor (PDGF)-dependent association of phospholipase C- γ with the PDGF receptor signaling complex. *Mol. Cell. Biol.* 10: 2359-2366.
4. Cantley, L.C., et al. 1991. Oncogenes and signal transduction. *Cell* 64: 281-302.
5. Koch, C.A., et al. 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* 252: 669-674.
6. Ravichandran, K.S., et al. 1993. Interaction of Shc with the ζ chain of the T cell receptor upon T cell activation. *Science* 262: 902-905.
7. Liu, S.K. and McGlade, C.J. 1998. Gads is a novel SH2 and SH3 domain-containing adaptor protein that binds to tyrosine-phosphorylated Shc. *Oncogene* 17: 3073-3082.

CHROMOSOMAL LOCATION

Genetic locus: GRAP2 (human) mapping to 22q13.1.

PRODUCT

Gads 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 Gads shRNA Plasmid (h): sc-40958-SH and Gads shRNA (h) Lentiviral Particles: sc-40958-V as alternate gene silencing products.

For independent verification of Gads (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-40958A, sc-40958B and sc-40958C.

RESEARCH USE

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

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

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

Gads (UW40): sc-73652 is recommended as a control antibody for monitoring of Gads 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 Gads gene expression knockdown using RT-PCR Primer: Gads (h)-PR: sc-40958-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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