

RhoGEF p115 siRNA (h): sc-41734

BACKGROUND

The Ras superfamily of GTPases, which can be subdivided into the Ras, Rho/Rac, Sar, Rab, ARF and Ran subfamilies, controls multiple aspects of cell function, including cytoskeletal rearrangement, nuclear signaling and cell growth. The Ras superfamily of GTPases function as regulated switches that toggle between a biologically active GTP-bound and an inactive GDP-bound form. This activation is catalyzed by guanine nucleotide exchange factors (GEFs). The Dbl-related proteins are a large family of structurally related molecules that have a common ability to catalyze GEF activity for specific members of the Ras family. Dbl-related proteins include FGD1, Lsc, RhoGEF p115, Lfc, Lbc and Brx. Lsc, Lbc and Lfc share sequence homology and show exchange activity toward Rho family GTPases. RhoGEF p115 catalyzes GEF activity for Rho but not Rac, Cdc42 or Ras GTPases.

REFERENCES

1. Bourne, H.R., et al. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125-132.
2. Boguski, M.S., et al. 1993. Proteins regulating Ras and its relatives. *Nature* 366: 643-654.
3. Whitehead, I.P., et al. 1996. Expression cloning of Lsc, a novel oncogene with structural similarities to the Dbl family of guanine nucleotide exchange factors. *J. Biol. Chem.* 271: 18643-18650.
4. Cerione, R.A., et al. 1996. The Dbl family of oncogenes. *Curr. Opin. Cell Biol.* 8: 216-222.

CHROMOSOMAL LOCATION

Genetic locus: ARHGEF1 (human) mapping to 19q13.2.

PRODUCT

RhoGEF p115 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 RhoGEF p115 shRNA Plasmid (h): sc-41734-SH and RhoGEF p115 shRNA (h) Lentiviral Particles: sc-41734-V as alternate gene silencing products.

For independent verification of RhoGEF p115 (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-41734A, sc-41734B and sc-41734C.

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

RhoGEF p115 siRNA (h) is recommended for the inhibition of RhoGEF p115 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

RhoGEF p115 (C-9): sc-74565 is recommended as a control antibody for monitoring of RhoGEF p115 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 RhoGEF p115 gene expression knockdown using RT-PCR Primer: RhoGEF p115 (h)-PR: sc-41734-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bodmann, E.L., et al. 2017. Potentiation of receptor responses induced by prolonged binding of G α_{13} and leukemia-associated RhoGEF. *FASEB J.* 31: 3663-3676.

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.