SANTA CRUZ BIOTECHNOLOGY, INC.

GRP1 siRNA (h): sc-40472

BACKGROUND

GRP1 (general receptor for phosphoinositides-1) contains a Pleckstrin homology (PH) domain as well as a Sec7 domain. The PH domain has high binding affinity for phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3), while the Sec7 homology domain is responsible for catalyzing guanine nucleotide exchange of ADP-ribosylation factor (ARF) proteins. GRP1 co-localizes with ARF6 and catalyzes GTP/GDP exchange on ARF6. It is known to interact with PtdIns(3,4,5)P3 localized to the plasma membrane *in vitro* and may also be a PtdIns(3,4,5)P3 receptor. Additionally, GRP1 may regulate protein sorting and membrane trafficking through interaction with the guanosine triphosphate ARF, and may control cell adhesion through interaction with integrins.

REFERENCES

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2. Klarlund, J.K., Rameh, L.E., Cantley, L.C., Buxton, J.M., Holik, J.J., Sakelis, C., Patki, V., Corvera, S. and Czech, M.P. 1998. Regulation of GRP1-catalyzed ADP ribosylation factor guanine nucleotide exchange by phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. 273: 1859-1862.
3. Venkateswarlu. K., Gunn-Moore, F., Oatey, P.B., Tavare, J.M. and Cullen, P.J. 1998. Nerve growth factor- and epidermal growth factor-stimulated translocation of the ADP-ribosylation factor-exchange factor GRP1 to the plasma membrane of PC12 cells requires activation of phosphatidylinositol 3-kinase and the GRP1 Pleckstrin homology domain. Biochem. J. 335: 139-146.
4. Langille, S.E., Patki, V., Klarlund, J.K., Buxton, J.M., Holik, J.J., Chawla, A., Corvera, S. and Czech, M.P. 1999. ADP-ribosylation factor 6 as a target of guanine nucleotide exchange factor GRP1. J. Biol. Chem. 274: 27099-27104.

CHROMOSOMAL LOCATION

Genetic locus: PSCD3 (human) mapping to 7p22.1.

PRODUCT

GRP1 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 GRP1 shRNA Plasmid (h): sc-40472-SH and GRP1 shRNA (h) Lentiviral Particles: sc-40472-V as alternate gene silencing products.

For independent verification of GRP1 (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-40472A, sc-40472B and sc-40472C.

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 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

GRP1 siRNA (h) is recommended for the inhibition of GRP1 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

GRP1 (A-3): sc-271741 is recommended as a control antibody for monitoring of GRP1 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 GRP1 gene expression knockdown using RT-PCR Primer: GRP1 (h)-PR: sc-40472-PR (20 µl). 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.