SANTA CRUZ BIOTECHNOLOGY, INC.

cytohesin-1 siRNA (m): sc-40471

BACKGROUND

Cytohesin-1 is an ARF guanine nucleotide exchange factor (GEF). Cytohesin-1 contains a phospholipid-binding C-terminal pleckstrin homology (PH) domain, a central Sec7 domain and an N-terminal coiled-coil region. The PH domain binds to phosphatidylinositol 3,4,5-triphosphate (PtdIns-3,4,5-P3), a phosphatidylinositol 3-kinase (PI-3-kinase) metabolite. The Sec7 domain is responsible for GDP/GTP exchange activity and brefeldin A inhibition. Cytohesin-1 catalyzes *in vitro* nucleotide exchange on ARF1 and ARF3, but it has no effect on ARF6. Additionally, cytohesin-1 is a regulatory factor for the αLβ2 Integrin in lymphocytes. Through interaction with integrins, cytohesin-1 may participate in inside-out cell signaling.

REFERENCES

1. Liu, L. and Pohajdak, B. 1992. Cloning and sequencing of a human cDNA from cytolytic NK/T cells with homology to yeast Sec7. Biochim. Biophys. Acta 1132: 75-78.
2. Kolanus, W., et al. 1996. αLβ2 Integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule. Cell 86: 233-242.
3. Meacci, E., et al. 1997. Cytohesin-1, a cytosolic guanine nucleotide-exchange protein for ADP-ribosylation factor. Proc. Natl. Acad. Sci. USA 94: 1745-1748.
4. Klarlund, J.K., et al. 1997. Signaling by phosphoinositide-3,4,5-trisphosphate through proteins containing pleckstrin and Sec7 homology domains. Science 275: 1927-1930.
5. Sata, M., et al. 1998. Brefeldin A inhibited guanine nucleotide-exchange activity of Sec7 domain from yeast Sec7 with yeast and mammalian ADP ribosylation factors. Proc. Natl. Acad. Sci. USA 95: 4204-4208.
6. Franco, M., et al. 1998. ARNO3, a Sec7-domain guanine nucleotide exchange factor for ADP ribosylation factor 1, is involved in the control of Golgi structure and function. Proc. Natl. Acad. Sci. USA 95: 9926-9931.

CHROMOSOMAL LOCATION

Genetic locus: Cyth1 (mouse) mapping to 11 E2.

PRODUCT

cytohesin-1 siRNA (m) 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 cytohesin-1 shRNA Plasmid (m): sc-40471-SH and cytohesin-1 shRNA (m) Lentiviral Particles: sc-40471-V as alternate gene silencing products.

For independent verification of cytohesin-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40471A, sc-40471B and sc-40471C.

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

cytohesin-1 siRNA (m) is recommended for the inhibition of cytohesin-1 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 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

cytohesin-1/2 (D-11): sc-166542 is recommended as a control antibody for monitoring of cytohesin-1 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 cytohesin-1 gene expression knockdown using RT-PCR Primer: cytohesin-1 (m)-PR: sc-40471-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.