SANTA CRUZ BIOTECHNOLOGY, INC.

cytohesin-2 siRNA (m): sc-40469

BACKGROUND

Cytohesin-2, also known as ARNO (ARF nucleotide-binding-site opener), is an ARF-1 guanine nucleotide exchange factor (GEF). ARF (ADP Ribosylation factor) proteins, a group within the RAS superfamily, are GTP-binding proteins central to the process of vesicle budding. Cytohesin-2 contains an N-terminal coiled-coil domain, a Sec 7 domain responsible for GDP/GTP exchange activity, and a C-terminal pleckstrin homology (PH) domain responsible for binding to PIP2. GEF activity of cytohesin-2 is enhanced by binding of the PH domain to phosphatidylinositol 4,5-bisphosphate which recruits cytohesin-2 to membranes. Cytohesin-2 is localized to the plasma membrane in mammalian cells and *in vitro* cytohesin-2 stimulates nucleotide exchange on ARF1 and ARF6.

REFERENCES

1. Kahn, R.A. and Gilman, A.G. 1984. Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. J. Biol. Chem. 259: 6228-6234.
2. Moss, J. and Vaughan, M. 1995. Structure and function of ARF proteins: activators of cholera toxin and critical components of intracellular vesicular transport processes. J. Biol. Chem. 270: 12327-12330.
3. Chardin, P., Paris, S., Antonny, B., Robineau, S., Beraud-Dufour, S., Jackson, C.L. and Chabre, M. 1996. A human exchange factor for ARF contains Sec7 and pleckstrin-homology domains. Nature 384: 481-484.
4. Paris, S., Beraud-Dufour, S., Robineau, S., Bigay, J., Antonny, B., Chabre, M. and Chardin, P. 1997. Role of protein-phospholipid interactions in the activation of ARF-1 by the guanine nucleotide exchange factor ARNO. J. Biol. Chem. 272: 22221-22226.
5. Franco, M., Boretto, J., Sylviane, R., Monier, S., Goud, B., Chardin, P. and Chavrier, P. 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: 9929-9931.
6. Frank, S., Upender, S., Hansen, S. H. and Casanova, J.E.1998. ARNO is a guanine nucleotide exchange factor for ADP-ribosylation factor 6. J. Biol. Chem. 273: 23-27.

CHROMOSOMAL LOCATION

Genetic locus: Cyth2 (mouse) mapping to 7 B4.

PRODUCT

cytohesin-2 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-2 shRNA Plasmid (m): sc-40469-SH and cytohesin-2 shRNA (m) Lentiviral Particles: sc-40469-V as alternate gene silencing products.

For independent verification of cytohesin-2 (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-40469A, sc-40469B and sc-40469C.

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-2 siRNA (m) is recommended for the inhibition of cytohesin-2 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-2 (H-7): sc-374640 is recommended as a control antibody for monitoring of cytohesin-2 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-2 gene expression knockdown using RT-PCR Primer: cytohesin-2 (m)-PR: sc-40469-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.

PROTOCOLS

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