SANTA CRUZ BIOTECHNOLOGY, INC.

GRASP65 siRNA (h): sc-41228

BACKGROUND

The Golgi apparatus is a highly complex organelle comprised of a stack of cisternal membranes on the secretory pathway from the ER to the cell surface. The structure is maintained by an exoskeleton or Golgi matrix constructed from a family of coiled-coil protein, the golgins and other peripheral membrane components such as GRASP55 and GRASP65. GRASP55 (Golgi reassembly stacking protien or p59) is a component of the Golgi stacking machinery. GRASP55 is highly homologous to GRASP65 and contains two PDZ domains. GRASP55 is myristoylated and palmitoylated. Unlike GRASP65, GRASP55 does not have detectable binding with the vesicle docking protein GM130 and is located on the medial-Golgi rather than *cis*-Golgi. Both GRASP55 and GRASP65 function in the stacking of Golgi cisternae. The novel coiled-coil protein golgin 45 interacts with GRASP55 and the GTP form of Rab 2, suggesting that GRASP55 and golgin 45 form a Rab 2 effector complex on medial-Golgi essential for normal protein transport and Golgi structure. ERK2 directly phosphorylates GRASP55, which is phosphorylated in mitotic cells, suggesting that mitogen-activated protein kinase kinase (MKK)/ERK pathway phosphorylates the Golgi during mitosis.

REFERENCES

1. Shorter, J., et al. 1999. GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system. EMBO J. 18: 4949-4960.
2. Kuo, A., et al. 2000. Transmembrane transforming growth factor α tethers to the PDZ domain-containing, Golgi membrane-associated protein p59/GRASP55. EMBO J. 19: 6427-6439.

CHROMOSOMAL LOCATION

Genetic locus: GORASP1 (human) mapping to 3p22.2.

PRODUCT

GRASP65 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 GRASP65 shRNA Plasmid (h): sc-41228-SH and GRASP65 shRNA (h) Lentiviral Particles: sc-41228-V as alternate gene silencing products.

For independent verification of GRASP65 (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-41228A, sc-41228B and sc-41228C.

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

GRASP65 siRNA (h) is recommended for the inhibition of GRASP65 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

GRASP65 (D-12): sc-374423 is recommended as a control antibody for monitoring of GRASP65 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 GRASP65 gene expression knockdown using RT-PCR Primer: GRASP65 (h)-PR: sc-41228-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Rivero, S., et al. 2009. Microtubule nucleation at the *cis*-side of the Golgi apparatus requires AKAP450 and GM130. EMBO J. 28: 1016-1028.
2. Yang, H., et al. 2011. Insulin-like growth factor 1 activates methionine adenosyltransferase 2A transcription by multiple pathways in human colon cancer cells. Biochem. J. 436: 507-516.
3. Casey, C.A., et al. 2018. Giantin is required for post-alcohol recovery of Golgi in liver cells. Biomolecules 8:150.
4. Manca, S., et al. 2019. The role of alcohol-induced Golgi fragmentation for androgen receptor signaling in prostate cancer. Mol. Cancer Res. 17: 225-237.

RESEARCH USE

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