SANTA CRUZ BIOTECHNOLOGY, INC.

GRAP1 siRNA (h): sc-105416

BACKGROUND

GRAP1, also designated GRASP-1 or GRIP-associated protein contains a RasGEF (Ras GDP/GTP exchange factor) domain, a caspase-3 cleavage site, a region homologous to RBD (Ras binding domain), and a PDZ domain. The caspase-3 cleavage site separates these domains into an amino terminal GEF catalytic domain and a carboxyl-terminal regulatory domain, which is a proteolytic fragment. This overall structure is similar to ralGDS. GRAP1 is a member of RasGEF (Ras protein GDP/GTP exchange factors) family. GRAP1 is expressed in the cytosol and partially localized to the membrane in all tissues of the nervous system, while the fragment is located only in the cytosol. GRAP1 associates with the seventh GRIP-1 (glutamate receptor interacting protein) PDZ domain. GRIP-1 binds to the C termini of AMPA receptors and may be an adapter protein that links AMPA receptors to other proteins. GRAP1 may be involved in the regulation of Ras signaling and AMPA receptor distribution, through the activation of NMDA receptors. Caspase-3 may disrupt the proper regulation or targeting of GEF by cleaving the regulatory domain from the catalytic domain.

REFERENCES

1. Kikuchi, A., et al. 1994. RalGDS family members interact with the effector loop of Ras p21. Mol. Cell. Biol. 14: 7483-7491.
2. Spaargaren, M. and Bischoff, J.R. 1994. Identification of the guanine nucleotide dissociation stimulator for Ral as a putative effector molecule of R-Ras, H-Ras, K-Ras, and Rap. Proc. Natl. Acad. Sci. USA 91: 12609-12613.
3. Dong, H., et al. 1997. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature 386: 279-284.
4. Srivastava, S., et al. 1998. Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. Neuron 21: 581-591.
5. Dong, H., et al. 1999. Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. J. Neurosci. 19: 6930-6941.
6. Ye, B., et al. 2000. GRASP-1: a neuronal RasGEF associated with the AMPA receptor/GRIP complex. Neuron 26: 603-617.

CHROMOSOMAL LOCATION

Genetic locus: GRIPAP1 (human) mapping to Xp11.23.

PRODUCT

GRAP1 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 GRAP1 shRNA Plasmid (h): sc-105416-SH and GRAP1 shRNA (h) Lentiviral Particles: sc-105416-V as alternate gene silencing products.

For independent verification of GRAP1 (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-105416A, sc-105416B and sc-105416C.

RESEARCH USE

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

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

GRAP1 siRNA (h) is recommended for the inhibition of GRAP1 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

GRAP1 (A-6): sc-398198 is recommended as a control antibody for monitoring of GRAP1 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 GRAP1 gene expression knockdown using RT-PCR Primer: GRAP1 (h)-PR: sc-105416-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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