SANTA CRUZ BIOTECHNOLOGY, INC.

RasGRP3 siRNA (m): sc-61445

BACKGROUND

Members of the Ras subfamily of GTPases function in signal transduction as GTP/GDP-modulated switches that rotate between inactive GDP- and active GTP-bound states. Guanine nucleotide exchange factors (GEFs), such as RasGRP3 (GRP3), act as Ras activators by promoting retrieval of GTP to maintain the active GTP-bound state and are the fundamental link between cell surface receptors and Ras activation. Highest levels of RasGRP3 expression are observed in heart, brain, lung and kidney tissues, and intermediate expression is observed in liver, skeletal muscle, pancreas, spleen, testis and ovary tissues. RasGRP3, which shares significant sequence identity with the calcium- and diacylglycerol-activated GEFs, activates Ras and Rap 1 and promotes activation of ELK1 in prostate cancer cell lines.

REFERENCES

1. Nagase, T., et al. 1999. Prediction of the coding sequences of unidentified human genes. XII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 5: 355-364.
2. Rebhun, J.F., et al. 2000. Identification of guanine nucleotide exchange factors (GEFs) for the Rap 1 GTPase. Regulation of MR-GEF by M-Ras-GTP interaction. J. Biol. Chem. 275: 34901-34908.
3. Aiba, Y., et al. 2004. Activation of RasGRP3 by phosphorylation of Thr-133 is required for B cell receptor-mediated Ras activation. Proc. Natl. Acad. Sci. USA 101: 16612-16617.
4. Roberts, D.M., et al. 2004. A vascular gene trap screen defines RasGRP3 as an angiogenesis-regulated gene required for the endothelial response to phorbol esters. Mol. Cell. Biol. 24: 10515-10528.
5. Braun, D.C., et al. 2005. Role of phorbol ester localization in determining protein kinase C or RasGRP3 translocation: real-time analysis using fluorescent ligands and proteins. Mol. Cancer Ther. 4: 141-150.
6. Coughlin, J.J., et al. 2005. RasGRP1 and RasGRP3 regulate B cell proliferation by facilitating B cell receptor-Ras signaling. J. Immunol. 175: 7179-7184.
7. Ozaki, N., et al. 2005. RasGRP3 mediates phorbol ester-induced, protein kinase C-independent exocytosis. Biochem. Biophys. Res. Commun. 329: 765-771.

CHROMOSOMAL LOCATION

Genetic locus: Rasgrp3 (mouse) mapping to 17 E2.

PRODUCT

RasGRP3 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 RasGRP3 shRNA Plasmid (m): sc-61445-SH and RasGRP3 shRNA (m) Lentiviral Particles: sc-61445-V as alternate gene silencing products.

For independent verification of RasGRP3 (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-61445A, sc-61445B and sc-61445C.

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

RasGRP3 siRNA (m) is recommended for the inhibition of RasGRP3 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

RasGRP3 (H-1): sc-271068 is recommended as a control antibody for monitoring of RasGRP3 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 RasGRP3 gene expression knockdown using RT-PCR Primer: RasGRP3 (m)-PR: sc-61445-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.