SANTA CRUZ BIOTECHNOLOGY, INC.

GRF-1 siRNA (h): sc-97682

BACKGROUND

The glucocorticoid receptor (GR) is a ligand-dependent, transactivating regulatory protein that is a member of the nuclear receptor superfamily. GRF-1 (glucocorticoid receptor DNA-binding factor 1), also known as p190RhoGAP or simply p190, is a transcriptional regulator which binds to the promoter region of the glucocorticoid receptor gene and represses its expression. By repressing GR expression, GRF-1 acts to down-regulate Rho signaling, thereby mediating both actin cytoskeletal rearrangements and cell cycle events. Through its GAP domain, GRF-1 is thought to affect cytokinesis by regulating Rho activity; a regulation that is controlled by the ubiquination of the GTP binding region and subsequent degradation of GRF-1. Additionally, GRF-1 plays an important role in oligodendrocyte differentiation, a process that is absent in malignant glioma tumors, implicating GRF-1 as a possible tumor suppressor. GRF-1 expression is regulated by glucocorticoids and the expressed protein exists as two isoforms produced by alternative splicing events.

REFERENCES

1. Dib, K., et al. 2001. Role of p190RhoGAP in β 2 integrin regulation of RhoA in human neutrophils. J. Immunol. 166: 6311-6322.
2. Su, L., et al. 2003. p190RhoGAP is cell cycle regulated and affects cytokinesis. J. Cell Biol. 163: 571-582.
3. Hernández, S.E., et al. 2004. Adhesion-dependent regulation of p190RhoGAP in the developing brain by the Abl-related gene tyrosine kinase. Curr. Biol. 14: 691-696.
4. Holinstat, M., et al. 2006. Suppression of RhoA activity by focal adhesion kinase-induced activation of p190RhoGAP: role in regulation of endothelial permeability. J. Biol. Chem. 281: 2296-2305.
5. Sastry, S.K., et al. 2006. PTP-PEST couples membrane protrusion and tail retraction via VAV2 and p190RhoGAP. J. Biol. Chem. 281: 11627-11636.
6. Kusama, T., et al. 2006. Inactivation of Rho GTPases by p190 RhoGAP reduces human pancreatic cancer cell invasion and metastasis. Cancer Sci. 97: 848-853.

CHROMOSOMAL LOCATION

Genetic locus: ARHGAP35 (human) mapping to 19q13.32.

PRODUCT

GRF-1 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 GRF-1 shRNA Plasmid (h): sc-97682-SH and GRF-1 shRNA (h) Lentiviral Particles: sc-97682-V as alternate gene silencing products.

For independent verification of GRF-1 (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-97682A, sc-97682B and sc-97682C.

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

GRF-1 siRNA (h) is recommended for the inhibition of GRF-1 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

GRF-1 (C-4): sc-390997 is recommended as a control antibody for monitoring of GRF-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 GRF-1 gene expression knockdown using RT-PCR Primer: GRF-1 (h)-PR: sc-97682-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. Daubon, T., et al. 2016. VEGF-A stimulates podosome-mediated collagen-IV proteolysis in microvascular endothelial cells. J. Cell Sci. 129: 2586-2598.

RESEARCH USE

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