SANTA CRUZ BIOTECHNOLOGY, INC.

# GRAF siRNA (h): sc-60755

## BACKGROUND

Cellular signaling by G-proteins is downregulated by GTPase-activating proteins (GAPs), which increase the rate of GTP hydroylsis. The GTPase regulator associated with focal adhesion kinase (GRAF) has GAP activity toward Rho A and Cdc42, but not Rac 1. GRAF is ubiquitously expressed with high levels in heart and brain. Expression of GRAF causes clearing of stress fibers and formation of long Actin-based filopodial-like extensions. Fusion of MLL with GRAF, MLL/GRAF, is included in a rare genetic subgroup of acute myeloid leukemia (AML) cases.

## REFERENCES


1. Taylor, J.M., et al. 1998. Characterization of GRAF, the GTPase-activating protein for Rho associated with focal adhesion kinase. Phosphorylation and possible regulation by mitogen-activated protein kinase. J. Biol. Chem. 273: 8063-8070.
2. Taylor, J.M., et al. 1999. Cytoskeletal changes induced by GRAF, the GTPase regulator associated with focal adhesion kinase, are mediated by Rho. J. Cell Sci. 112: 231-242.
3. Sheffield, P.J., et al. 1999. Expression, purification and crystallization of a BH domain from the GTPase regulatory protein associated with focal adhesion kinase. Acta Crystallogr. D, Biol. Crystallogr. 55: 356-359.
4. Borkhardt, A., et al. 2000. The human GRAF gene is fused to MLL in a unique t(5;11)(q31;q23) and both alleles are disrupted in three cases of myelodysplastic syndrome/acute myeloid leukemia with a deletion 5q. Proc. Natl. Acad. Sci. USA 97: 9168-9173.
5. Longenecker, K.L., et al. 2001. Structure of the BH domain from GRAF and its implications for Rho GTPase recognition. J. Biol. Chem. 275: 38605-38610.
6. Shibata, H., et al. 2001. PKNβ interacts with the SH3 domains of GRAF and a novel GRAF related protein, GRAF2, which are GTPase activating proteins for Rho family. J. Biochem. 130: 23-31.
7. Panagopoulos, I., et al. 2004. MLL/GRAF fusion in an infant acute monocytic leukemia (AML M5b) with a cytogenetically cryptic ins(5;11)(q31;q23q23). Genes Chromosomes Cancer 41: 400-404.


## CHROMOSOMAL LOCATION

Genetic locus: ARHGAP26 (human) mapping to 5q31.3.

## PRODUCT

GRAF 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 GRAF shRNA Plasmid (h): sc-60755-SH and GRAF shRNA (h) Lentiviral Particles: sc-60755-V as alternate gene silencing products.

For independent verification of GRAF (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-60755A, sc-60755B and sc-60755C.

## 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

GRAF siRNA (h) is recommended for the inhibition of GRAF 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.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GRAF gene expression knockdown using RT-PCR Primer: GRAF (h)-PR: sc-60755-PR (20 µl, 416 bp). 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.