

# LARG siRNA (h): sc-41800

## BACKGROUND

Leukemia-associated RhoGEF (LARG) is a 1,544 amino acid, guanine nucleotide exchange factor (GEF) that contains a PDZ domain, a LH/RGS domain, and a Dbl homology/pleckstrin homology domain. LARG shares homology with other RhoGEFs, including such oncogenes as DBL, VAV1, TIAM, and BCR. RhoGEFs containing RGS domains are capable of associating with activated  $G_{\alpha}$  subunits, and can function as GTPase activating proteins (GAPs). LARG transcripts have been detected in human peripheral blood leukocytes, spleen, prostate, testis, ovary, small intestine, colon, and thymus. The LARG protein may elicit signals through a G protein-coupled receptor (GPCR)-Rho-dependent signaling pathway. Genetic alterations that occur at human chromosome position 11q23.3, where LARG maps, are prevalent in acute leukemias.

## REFERENCES

1. Nagase, T., et al. 1997. Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. DNA Res. 4: 141-150.
2. Kourlas, P.J., et al. 2000. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc. Natl. Acad. Sci. USA 97: 2145-2150.
3. Fukuhara, S., et al. 2000. Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G12 family to Rho. FEBS Lett. 485: 183-188.

## CHROMOSOMAL LOCATION

Genetic locus: ARHGEF12 (human) mapping to 11q23.3.

## PRODUCT

LARG 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LARG shRNA Plasmid (h): sc-41800-SH and LARG shRNA (h) Lentiviral Particles: sc-41800-V as alternate gene silencing products.

For independent verification of LARG (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-41800A, sc-41800B and sc-41800C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}$  C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

LARG siRNA (h) is recommended for the inhibition of LARG 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  $\mu$ M in 66  $\mu$ l. 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

LARG (H-3): sc-166318 is recommended as a control antibody for monitoring of LARG 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LARG gene expression knockdown using RT-PCR Primer: LARG (h)-PR: sc-41800-PR (20  $\mu$ l, 535 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.

## SELECT PRODUCT CITATIONS

1. Jiang, X., et al. 2010. HGAL, a germinal center specific protein, decreases lymphoma cell motility by modulation of the RhoA signaling pathway. Blood 116: 5217-5227.
2. Heemskerk, N., et al. 2016. F-Actin-rich contractile endothelial pores prevent vascular leakage during leukocyte diapedesis through local RhoA signalling. Nat. Commun. 7: 10493.
3. Bodmann, E.L., et al. 2017. Potentiation of receptor responses induced by prolonged binding of  $G_{\alpha 13}$  and leukemia-associated RhoGEF. FASEB J. 31: 3663-3676.
4. Zhang, F., et al. 2021. Reregulation of hepatic stellate cell contraction and cirrhotic portal hypertension by Wnt/ $\beta$ -catenin signaling via interaction with Gli1. Br. J. Pharmacol. 178: 378-380.

## RESEARCH USE

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