SANTA CRUZ BIOTECHNOLOGY, INC.

GRAIL siRNA (h): sc-90884

BACKGROUND

GRAIL, also known as ring finger protein 128, is a 428 amino acid type I transmembrane protein localized to the intracytoplasmic membrane. GRAIL contains a protease-associated (PA) domain and a RING finger domain, which binds to E2 ubiquitin-conjugating enzymes. When under anergic conditions, GRAIL functions as an E3 ubiquitin-protein ligase that inhibits IL-2, IL-4 and various other cytokines. GRAIL is also thought to be involved in the patterning of the dorsal ectoderm during development. Expressed in an asymmetric perinuclear punctate manner, GRAIL colocalizes with Rab 7, GRP 78 and Syntaxin 5. GRAIL is expressed as two isoforms produced by alternative splicing.

REFERENCES

1. Anandasabapathy, N., et al. 2003. GRAIL: an E3 ubiquitin ligase that inhibits cytokine gene transcription is expressed in anergic $CD4^+$ T cells. Immunity 18: 535-547.
2. Soares, L., et al. 2004. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. Nat. Immunol. 5: 45-54.
3. Su, L., et al. 2006. A novel E3 ubiquitin ligase substrate screen identifies Rho guanine dissociation inhibitor as a substrate of gene related to anergy in lymphocytes. J. Immunol. 177: 7559-7566.
4. MacKenzie, D.A., et al. 2007. GRAIL is up-regulated in $CD4^+$ $CD25^+$ T regulatory cells and is sufficient for conversion of T cells to a regulatory phenotype. J. Biol. Chem. 282: 9696-9702.
5. Kostianovsky, A.M., et al. 2007. Up-regulation of gene related to anergy in lymphocytes is associated with Notch-mediated human T cell suppression. J. Immunol. 178: 6158-6163.
6. Egawa, S., et al. 2008. Upregulation of GRAIL is associated with remission of ulcerative colitis. Am. J. Physiol. Gastrointest. Liver Physiol. 295: G163-G169.

CHROMOSOMAL LOCATION

Genetic locus: RNF128 (human) mapping to Xq22.3.

PRODUCT

GRAIL 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 GRAIL shRNA Plasmid (h): sc-90884-SH and GRAIL shRNA (h) Lentiviral Particles: sc-90884-V as alternate gene silencing products.

For independent verification of GRAIL (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-90884A, sc-90884B and sc-90884C.

PROTOCOLS

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

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

GRAIL siRNA (h) is recommended for the inhibition of GRAIL 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 GRAIL gene expression knockdown using RT-PCR Primer: GRAIL (h)-PR: sc-90884-PR (20 µl, 433 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.