

# G-CSF siRNA (h): sc-39389

## BACKGROUND

Granulocyte-colony stimulating factor, G-CSF, is a pleiotropic cytokine that influences differentiation, proliferation and activation of the neutrophilic granulocyte lineage. The murine G-CSF cDNA encodes a 208 amino acid precursor containing a 30 amino acid signal peptide that is proteolytically cleaved to form a 178 amino acid residue mature protein. Two G-CSF cDNAs which are identical except for a three amino acid deletion in the amino terminus of one form of the protein have been isolated from human cells. Murine and human G-CSF share 73% sequence identity at the amino acid level. G-CSF signals through the G-CSF receptor, G-CSFR, a heavily glycosylated 812 amino acid polypeptide with a single transmembrane domain. Stimulation of the G-CSFR results in the activation of the Ras/MAPK pathway and phosphorylation of the adaptor protein Shc. Other studies indicate that the kinases Lyn and Syk and the transcription factor Stat3 are activated in response to G-CSF stimulation.

## REFERENCES

1. Tsuchiya, M., et al. 1986. Isolation and characterization of the cDNA for murine granulocyte colony-stimulating factor. *Proc. Natl. Acad. Sci.* 83: 7633-7637.
2. Nagata, S., et al. 1986. Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature* 319: 415-418.
3. Abrams, J.S., et al. 1992. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol. Rev.* 127: 5-24.
4. Visani, G., et al. 1995. G-CSF in the biology and treatment of acute myeloid leukemias. *Leuk. Lymphoma* 18: 423-428.

## CHROMOSOMAL LOCATION

Genetic locus: CSF3 (human) mapping to 17q21.1.

## PRODUCT

G-CSF 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 G-CSF shRNA Plasmid (h): sc-39389-SH and G-CSF shRNA (h) Lentiviral Particles: sc-39389-V as alternate gene silencing products.

For independent verification of G-CSF (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-39389A, sc-39389B and sc-39389C.

## 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

G-CSF siRNA (h) is recommended for the inhibition of G-CSF 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

G-CSF (3D1): sc-53292 is recommended as a control antibody for monitoring of G-CSF 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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor G-CSF gene expression knockdown using RT-PCR Primer: G-CSF (h)-PR: sc-39389-PR (20  $\mu$ l). 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. Venkatasubramanian, S., et al. 2015. A rho GDP dissociation inhibitor produced by apoptotic T-cells inhibits growth of *Mycobacterium tuberculosis*. *PLoS Pathog.* 11: e1004617.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.