

GLI-3 siRNA (h): sc-35483

BACKGROUND

Zinc-finger proteins contain DNA-binding domains and have a wide variety of functions, most of which encompass some form of transcriptional activation or repression. The majority of zinc-finger proteins contain a Krüppel-type DNA binding domain and a KRAB domain, which is thought to interact with KAP1, thereby recruiting histone modifying proteins. GLI-3 (GLI family zinc finger 3), also known as GLI3FL (GLI3 full length protein), PHS, ACLS, GCPS, PAPA, PAPB, PAPA1 or PPDIV, is a 1,580 amino acid nuclear and cytoplasmic protein that acts as both a transcriptional activator and a repressor of the Sonic hedgehog (Shh) pathway. A member of the GLI C₂H₂-type zinc-finger protein family, GLI-3 is encoded by a gene that maps to human chromosome 7p14.1. Defects in the GLI-3 gene are the cause of a disorder known as Greig cephalo-poly-syndactyly syndrome (GCPS), which affects limb and craniofacial development.

REFERENCES

1. Ruppert, J.M., et al. 1988. The GLI-Krüppel family of human genes. *Mol. Cell. Biol.* 8: 3104-3113.
2. Ruppert, J.M., et al. 1990. GLI3 encodes a 190-kilodalton protein with multiple regions of GLI similarity. *Mol. Cell. Biol.* 10: 5408-5415.
3. Wild, A., et al. 1997. Point mutations in human GLI3 cause Greig syndrome. *Hum. Mol. Genet.* 6: 1979-1984.
4. Kalfs-Suske, M., et al. 1999. Point mutations throughout the GLI3 gene cause Greig cephalopolysyndactyly syndrome. *Hum. Mol. Genet.* 8: 1769-1777.

CHROMOSOMAL LOCATION

Genetic locus: GLI3 (human) mapping to 7p14.1.

PRODUCT

GLI-3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GLI-3 shRNA Plasmid (h): sc-35483-SH and GLI-3 shRNA (h) Lentiviral Particles: sc-35483-V as alternate gene silencing products.

For independent verification of GLI-3 (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-35483A, sc-35483B and sc-35483C.

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

APPLICATIONS

GLI-3 siRNA (h) is recommended for the inhibition of GLI-3 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 μ M in 66 μ 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

GLI-3 (B-4): sc-74478 is recommended as a control antibody for monitoring of GLI-3 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 GLI-3 gene expression knockdown using RT-PCR Primer: GLI-3 (h)-PR: sc-35483-PR (20 μ l, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Deb Pal, A., et al. 2015. Epstein-Barr virus latent membrane protein 2A mediated activation of Sonic hedgehog pathway induces HLA class Ia downregulation in gastric cancer cells. *Virology* 484: 22-32.

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.