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EXT1 siRNA (m): sc-144984

BACKGROUND

Hereditary multiple exostoses (HME) is an autosomal dominant disorder characterized by the formation of exostoses (EXT), which are cartilage-capped bony protuberances mainly located on long bones. Two proteins associated with EXT, EXT1 and EXT2, form homo/heteromeric complexes *in vivo*, which leads to the accumulation of both proteins in the Golgi apparatus. EXT1 and EXT2 are endoplasmic reticulum-localized type II transmembrane glycoproteins that possess, or are tightly associated with, glycosyltransferase activities involved in the polymerization of the glycosaminoglycan, heparan sulfate (HS). EXT2 is a protein that harbors the D-glucuronyl (GlcA) and N-acetyl-D-glucosaminyl (GlcNAc) transferase activities required for biosynthesis of HS. EXT1 rescues defective HS biosynthesis and elevates low GlcA and GlcNAc transferase levels in mutated cells.

REFERENCES

1. Lind, T., et al. 1998. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J. Biol. Chem.* 273: 26265-26268.
2. McCormick, C., et al. 1998. The putative tumour suppressor EXT1 alters the expression of cell-surface heparan sulfate. *Nat. Genet.* 19: 158-161.
3. Wuyts, W. and Van Hul, W. 2000. Molecular basis of multiple exostoses: mutations in the EXT1 and EXT2 genes. *Hum. Mutat.* 15: 220-2277.
4. Kobayashi, S., et al. 2000. Association of EXT1 and EXT2, hereditary multiple exostoses gene products, in Golgi apparatus. *Biochem. Biophys. Res. Commun.* 268: 860-867.
5. McCormick, C., et al. 2000. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc. Natl. Acad. Sci. USA* 97: 668-673.

CHROMOSOMAL LOCATION

Genetic locus: Ext1 (mouse) mapping to 15 C.

PRODUCT

EXT1 siRNA (m) 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 EXT1 shRNA Plasmid (m): sc-144984-SH and EXT1 shRNA (m) Lentiviral Particles: sc-144984-V as alternate gene silencing products.

For independent verification of EXT1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-144984A, sc-144984B and sc-144984C.

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 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

EXT1 siRNA (m) is recommended for the inhibition of EXT1 expression in mouse 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

EXT1 (A-7): sc-515144 is recommended as a control antibody for monitoring of EXT1 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 EXT1 gene expression knockdown using RT-PCR Primer: EXT1 (m)-PR: sc-144984-PR (20 μ l). 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.