

UGGT1 siRNA (r): sc-60097

BACKGROUND

UDP-glucose glycoprotein glucosyltransferase 1 (UGGT1 or HUGT1), belongs to the glycosyltransferase 8 family of proteins. UGGT1 is involved in glycosylation pathways and induced by tunicamycin and A23187. Its main function is to recognize glycoproteins with folding defects. It reglucosylates single N-glycans near the misfolded area, flagging these proteins for recycling to the endoplasmic reticulum (ER) followed by refolding or degradation. UGGT1, which localizes to the ER and to the ER-Golgi intermediate compartment, is primarily expressed in skeletal muscle, pancreas, brain and kidney tissues.

REFERENCES

1. Amouzadeh, H.R., et al. 1997. UDP-glucose glycoprotein glucosyltransferase associates with endoplasmic reticulum chaperones and its activity is decreased *in vivo* by the inhalation anesthetic halothane. *Chem. Res. Toxicol.* 10: 59-63.
2. Arnold, S.M., et al. 2000. Two homologues encoding human UDP-glucose glycoprotein glucosyltransferase differ in mRNA expression and enzymatic activity. *Biochemistry* 39: 2149-2163.
3. Tessier, D.C., et al. 2000. Cloning and characterization of mammalian UDP-glucose glycoprotein glucosyltransferase and the development of a specific substrate for this enzyme. *Glycobiology* 10: 403-412.
4. Dejgaard, S., et al. 2003. The ER glycoprotein quality control system. *Curr. Issues Mol. Biol.* 6: 29-42.
5. Taylor, S.C., et al. 2003. Glycopeptide specificity of the secretory protein folding sensor UDP-glucose glycoprotein glucosyltransferase. *EMBO Rep.* 4: 405-411.
6. Taylor, S.C., et al. 2004. The ER protein folding sensor UDP-glucose glycoprotein glucosyltransferase modifies substrates distant to local changes in glycoprotein conformation. *Nat. Struct. Mol. Biol.* 11: 128-134.
7. Ito, Y., et al. 2005. Structural approaches to the study of oligosaccharides in glycoprotein quality control. *Curr. Opin. Struct. Biol.* 15: 481-489.

CHROMOSOMAL LOCATION

Genetic locus: Ugg1 (rat) mapping to 9q21.

PRODUCT

UGGT1 siRNA (r) 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 UGGT1 shRNA Plasmid (r): sc-60097-SH and UGGT1 shRNA (r) Lentiviral Particles: sc-60097-V as alternate gene silencing products.

For independent verification of UGGT1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60097A, sc-60097B and sc-60097C.

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

UGGT1 siRNA (r) is recommended for the inhibition of UGGT1 expression in rat 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

UGGT1 (H-9): sc-374565 is recommended as a control antibody for monitoring of UGGT1 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 UGGT1 gene expression knockdown using RT-PCR Primer: UGGT1 (r)-PR: sc-60097-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.