

O-GlcNAc transferase siRNA (h): sc-40780

BACKGROUND

O-linked N-acetylglucosamine (O-GlcNAc) transferase (also designated OGT) catalyzes the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues. Since both phosphorylation and glycosylation compete for similar serine or threonine residues, the two processes may compete for sites, or they may alter the substrate specificity of nearby sites by steric or electrostatic effects. O-GlcNAc transferase has been purified from rat liver. It exists as a heterotrimeric complex with two subunits of the same molecular mass and one shorter subunit. Both polypeptides are related; the short subunit band is either a proteolytic product of the polypeptide or the product of an alternative translation start site. O-GlcNAc transferase is expressed as multiple transcripts that are present in different amounts in various human tissues, with the highest levels of expression in pancreas. Immunofluorescence of human cells expressing rat O-GlcNAc transferase indicated that it is present in both the nucleus and cytosol. HeLa cells expressing O-GlcNAc transferase do not survive well during prolonged incubations, suggesting that this protein may be toxic to the cells.

CHROMOSOMAL LOCATION

Genetic locus: OGT (human) mapping to Xq13.1.

PRODUCT

O-GlcNAc transferase 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 O-GlcNAc transferase shRNA Plasmid (h): sc-40780-SH and O-GlcNAc transferase shRNA (h) Lentiviral Particles: sc-40780-V as alternate gene silencing products.

For independent verification of O-GlcNAc transferase (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-40780A, sc-40780B and sc-40780C.

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

O-GlcNAc transferase siRNA (h) is recommended for the inhibition of O-GlcNAc transferase expression in human cells.

PROTOCOLS

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

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

O-GlcNAc transferase (F-12): sc-74546 is recommended as a control antibody for monitoring of O-GlcNAc transferase 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor O-GlcNAc transferase gene expression knockdown using RT-PCR Primer: O-GlcNAc transferase (h)-PR: sc-40780-PR (20 μ l, 465 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

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- Kang, K.A., et al. 2016. Interaction of DNA demethylase and histone methyltransferase upregulates Nrf2 in 5-fluorouracil-resistant colon cancer cells. *Oncotarget* 7: 40594-40620.
- Pepe, F., et al. 2017. Regulation of miR-483-3p by the O-linked N-acetylglucosamine transferase links chemosensitivity to glucose metabolism in liver cancer cells. *Oncogenesis* 6: e328.
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- Lafont, F., et al. 2020. DNA-PK_{CS} Ser2056 auto-phosphorylation is affected by an O-GlcNAcylation/phosphorylation interplay. *Biochim. Biophys. Acta Gen. Subj.* 1864: 129705.
- Huang, H., et al. 2021. O-GlcNAcylation promotes the migratory ability of hepatocellular carcinoma cells via regulating FOXA2 stability and transcriptional activity. *J. Cell. Physiol.* 236: 7491-7503.

RESEARCH USE

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