SANTA CRUZ BIOTECHNOLOGY, INC.

O-GlcNAc transferase siRNA (r): sc-156078

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.

REFERENCES

1. Haltiwanger, R.S., et al. 1992. Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine:polypeptide β-N-acetylglucosaminyltransferase. J. Biol. Chem. 267: 9005-9013.
2. Kreppel, L.K., et al. 1997. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J. Biol. Chem. 272: 9308-9315.

CHROMOSOMAL LOCATION

Genetic locus: Ogt (rat) mapping to Xq22.

PRODUCT

O-GlcNAc transferase 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see O-GlcNAc transferase shRNA Plasmid (r): sc-156078-SH and O-GlcNAc transferase shRNA (r) Lentiviral Particles: sc-156078-V as alternate gene silencing products.

For independent verification of O-GlcNAc transferase (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-156078A, sc-156078B and sc-156078C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

O-GlcNAc transferase siRNA (r) is recommended for the inhibition of O-GlcNAc transferase 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 µM in 66 µl. 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).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 O-GlcNAc transferase gene expression knockdown using RT-PCR Primer: O-GlcNAc transferase (r)-PR: sc-156078-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shin, S.J., et al. 2020. Disruption of retinoid homeostasis induces RBP4 overproduction in diabetes: O-GlcNAcylation involved. Metabolism 113: 154403.

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.