SANTA CRUZ BIOTECHNOLOGY, INC.

NCOAT siRNA (m): sc-62668

BACKGROUND

NCOAT (nuclear cytoplasmic O-GlcNAcase and acetyltransferase), also known as MGEA5 (meningioma-expressed antigen 5), HEXC or MEA5, is a bifunctional enzyme that functions as both a β-hexosaminidase and a histone acetyltransferase. Expressed ubiquitously with highest expression in placenta, brain and pancreas, NCOAT functions as a glycosidase that catalyzes the cleavage of O-GlcNAc residues from GlcNAc-modified proteins. In addition, NCOAT acetylates specific residues on Histone H3 and Histone H4, suggesting an important role in the histone code. The enzymatic activity of NCOAT is optimal at a slightly acidic pH of 5.7-7 and NCOAT function is competitively inhibited by free N-acetylglucosamine. Due to alternative splicing events, NCOAT is expressed as three isoforms. Isoform 1 localizes to the cytoplasm, while isoform 3 localizes to the nucleus.

REFERENCES

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2. Farook, V.S., et al. 2002. Analysis of MGEA5 on 10q24.1-q24.3 encoding the β-O-linked N-acetylglucosaminidase as a candidate gene for type 2 diabetes mellitus in Pima Indians. Mol. Genet. Metab. 77: 189-193.
3. Wells, L., et al. 2002. Dynamic O-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic β-N-acetylglucosaminidase, O-GlcNAcase. J. Biol. Chem. 277: 1755-1761.
4. Van Tine, B.A., et al. 2003. Assignment of N-acetyl-D-glucosaminidase (Mgea5) to rat chromosome 1q5 by tyramide fluorescence *in situ* hybridization (T-FISH): synteny between rat, mouse and human with Insulin degradation enzyme (IDE). Cytogenet. Genome Res. 103: 202B.
5. Lehman, D.M., et al. 2005. A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-acetyl-β-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes 54: 1214-1221.
6. Gao, F., et al. 2005. Hypoxia-induced alterations in hyaluronan and hyaluronidase. Adv. Exp. Med. Biol. 566: 249-256.
7. Whisenhunt, T.R., et al. 2006. Disrupting the enzyme complex regulating O-GlcNAcylation blocks signaling and development. Glycobiology 16: 551-563.
8. Toleman, C., et al. 2006. Location and characterization of the O-GlcNAcase active site. Biochim. Biophys. Acta 1760: 829-839.
9. Toleman, C.A., et al. 2006. The histone acetyltransferase NCOAT contains a zinc finger-like motif involved in substrate recognition. J. Biol. Chem. 281: 3918-3925.

CHROMOSOMAL LOCATION

Genetic locus: Mgea5 (mouse) mapping to 19 C3.

PROTOCOLS

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

PRODUCT

NCOAT 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NCOAT shRNA Plasmid (m): sc-62668-SH and NCOAT shRNA (m) Lentiviral Particles: sc-62668-V as alternate gene silencing products.

For independent verification of NCOAT (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-62668A, sc-62668B and sc-62668C.

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

NCOAT siRNA (m) is recommended for the inhibition of NCOAT 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NCOAT gene expression knockdown using RT-PCR Primer: NCOAT (m)-PR: sc-62668-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. Rahman, M.A., et al. 2019. Modulation of O-GlcNAcylation regulates autophagy in cortical astrocytes. Oxid. Med. Cell. Longev. 2019: 6279313.

RESEARCH USE

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