SANTA CRUZ BIOTECHNOLOGY, INC.

ADO siRNA (h): sc-90328

BACKGROUND

Human thiol dioxygenases include ADO (2-aminoethanethiol (cysteamine) dioxygenase) and CDO (cysteine dioxygenase). ADO adds two oxygen atoms to free cysteamine to form hypotaurine, whereas CDO adds two oxygen atoms to free cysteine. Encoded by a gene that maps to human chromosome 10q21.3, ADO is a 270 amino acid protein that is ubiquitously expressed, with highest levels in brain, heart and skeletal muscle. ADO is responsible for endogenous cysteamine dioxygenase activity and participates in metal ion binding, with iron as a cofactor. Overexpression of ADO in Hep G2/C3A cells increases production of hypotaurine from cysteamine. Conversely, reduced expression of ADO decreases hypotaurine production.

REFERENCES

1. Gianfrancesco, F., et al. 2004. Emergence of Talanin protein associated with human uric acid nephrolithiasis in the Hominidae lineage. Gene 339: 131-138.
2. Castermans, D., et al. 2007. Identification and characterization of the TRIP8 and REEP3 genes on chromosome 10q21.3 as novel candidate genes for autism. Eur. J. Hum. Genet. 15: 422-431.
3. Dominy, J.E., et al. 2007. Discovery and characterization of a second mammalian thiol dioxygenase, cysteamine dioxygenase. J. Biol. Chem. 282: 25189-25198.
4. Rioux, J.D., et al. 2007. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat. Genet. 39: 596-604.
5. Chin, M.H., et al. 2008. Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. J. Proteome Res. 7: 666-677.
6. Ueki, I. and Stipanuk, M.H. 2009. 3T3-L1 adipocytes and rat adipose tissue have a high capacity for taurine synthesis by the cysteine dioxygenase/cysteinesulfinate decarboxylase and cysteamine dioxygenase pathways. J. Nutr. 139: 207-214.
7. Stipanuk, M.H., et al. 2010. Thiol dioxygenases: unique families of cupin proteins. Amino Acids 41: 91-102.

CHROMOSOMAL LOCATION

Genetic locus: ADO (human) mapping to 10q21.3.

PRODUCT

ADO 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ADO shRNA Plasmid (h): sc-90328-SH and ADO shRNA (h) Lentiviral Particles: sc-90328-V as alternate gene silencing products.

For independent verification of ADO (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-90328A, sc-90328B and sc-90328C.

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

ADO siRNA (h) is recommended for the inhibition of ADO expression in human 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

ADO (E-11): sc-515318 is recommended as a control antibody for monitoring of ADO 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 ADO gene expression knockdown using RT-PCR Primer: ADO (h)-PR: sc-90328-PR (20 µl). 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.

PROTOCOLS

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