

ADK siRNA (h): sc-38902

BACKGROUND

Adenosine kinase (ATP:adenosine 5'-phosphotransferase), or ADK, is an abundant enzyme in mammalian tissues that catalyzes the transfer of the γ -phosphate from ATP to adenosine, thereby serving as a regulator of concentrations of both extracellular adenosine and intracellular adenine nucleotides. Adenosine, an extracellular signaling molecule, has widespread effects on the cardiovascular, nervous, respiratory, and immune systems with increased concentration at sites of tissue injury and inflammation. Adenosine is an efficient inhibitor of neuronal activity with the ability to suppress seizure activity in various animal models of epilepsy. The human ADK gene maps to chromosome 10q22.2 and encodes two ADK transcripts that encode a 345-amino acid form and a 362-amino acid form of the enzyme. These 2 alternately spliced forms differ only at the 5' end, where the first 4 encoded residues of the short form are replaced by 21 residues in the long form. When expressed, both isoforms of the enzyme phosphorylate adenosine with identical kinetics and both require Mg^{2+} for activity. ADK is fully active under dilute conditions, but tends to form soluble aggregates at higher concentrations, which results in inactivation of the enzyme.

REFERENCES

1. Sakowicz, M., et al. 2001. Expression level of adenosine kinase in rat tissues. Lack of phosphate effect on the enzyme activity. *Acta Biochim. Pol.* 48: 745-754.
2. Zumsteg, V., et al. 2002. The use of real-time PCR with fluorogenic probes for the rapid selection of mutant neuroectodermal grafts. *J. Neurosci. Methods* 120: 85.

CHROMOSOMAL LOCATION

Genetic locus: ADK (human) mapping to 10q22.2.

PRODUCT

ADK 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 ADK shRNA Plasmid (h): sc-38902-SH and ADK shRNA (h) Lentiviral Particles: sc-38902-V as alternate gene silencing products.

For independent verification of ADK (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-38902A, sc-38902B and sc-38902C.

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

ADK siRNA (h) is recommended for the inhibition of ADK 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 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

ADK (H-1): sc-514588 is recommended as a control antibody for monitoring of ADK 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 MarkerTM 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 ADK gene expression knockdown using RT-PCR Primer: ADK (h)-PR: sc-38902-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Chen, C., et al. 2019. 5'-iodotubercidin represses Insulinoma associated-1 expression, decreases cAMP levels, and suppresses human neuroblastoma cell growth. *J. Biol. Chem.* 294: 5456-5465.
2. Cao, W., et al. 2019. Adenosine kinase inhibition protects against cisplatin-induced nephrotoxicity. *Am. J. Physiol. Renal Physiol.* 317: F107-F115.
3. Cao, W., et al. 2020. Adenosine kinase inhibition attenuates ischemia reperfusion-induced acute kidney injury. *Life Sci.* 256: 117972.

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.