

AK3 siRNA (h): sc-29656

BACKGROUND

Adenylate kinases 1-5 (designated AK1-5) are a set of enzymes that regulate the phosphorylation state of intracellular adenine nucleotides, which are the principle high-energy phosphoryl-carrying molecules in living cells. AKs influence metabolic signals, which include gene expression, ion channel activity and protein kinase-mediated signaling, by catalyzing phosphoryl transfer between adenine nucleotides (AMP, ADP, ATP). Inherited mutations leading to AK deficiencies in erythrocytes have been implicated in hemolytic anemia. Human AK3 is a 223 amino acid protein that is present in the mitochondria of liver and heart and utilizes GTP as a substrate relative to isoforms AK1 and AK2, which use ATP.

REFERENCES

1. Shahjahan, M., et al. 1991. Cloning and characterization of the gene encoding bovine mitochondrial adenylate kinase isozyme 3. *Gene* 107: 313-317.
2. Xu, G., et al. 1992. Characterization of human adenylate kinase 3 (AK3) cDNA and mapping of the AK3 pseudogene to an intron of the NF1 gene. *Genomics* 13: 537-542.
3. Barile, M., et al. 1994. Mechanisms of toxicity of 3'-azido-3'-deoxythymidine. Its interaction with adenylate kinase. *Biochem. Pharmacol.* 48: 1405-1412.
4. Dzeja, P.P., et al. 1998. Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes. *Mol. Cell. Biochem.* 184: 169-182.
5. Noma, T., et al. 1999. Characterization of the 5'-flanking region of the gene encoding bovine adenylate kinase isozyme 3. *Biochim. Biophys. Acta* 1489: 383-388.
6. Noma, T., et al. 1999. Cloning and functional characterization of the promoter region of the gene encoding human adenylate kinase isozyme 3. *Biochem. Biophys. Res. Commun.* 264: 990-997.
7. Online Mendelian Inheritance in Man, OMIM[™]. 1999. Johns Hopkins University, Baltimore, MD. MIM Number: 103000. World Wide Web URL. <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: AK3L1 (human) mapping to 9p24.1.

PRODUCT

AK3 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 AK3 shRNA Plasmid (h): sc-29656-SH and AK3 shRNA (h) Lentiviral Particles: sc-29656-V as alternate gene silencing products.

For independent verification of AK3 (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-29656A, sc-29656B and sc-29656C.

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

AK3 siRNA (h) is recommended for the inhibition of AK3 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

AK3 (E-5): sc-398571 is recommended as a control antibody for monitoring of AK3 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 Marker[™] 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 AK3 gene expression knockdown using RT-PCR Primer: AK3 (h)-PR: sc-29656-PR (20 μ l). 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.