SANTA CRUZ BIOTECHNOLOGY, INC.

ADE2 siRNA (h): sc-105042

BACKGROUND

Two successive steps in *de novo* purine biosynthesis are catalyzed by the enzymes 5-aminoimidazole ribonucleotide (AIR) carboxylase and 4-[(N-succinylamino)carbonyl]-5-aminoimidazole ribonucleotide (SAICAR) synthetase. In vertebrates, a bifunctional enzyme, ADE2 (also designated PurCE/AIRC) catalyzes successive carboxylation and aspartylation steps of AIR to form SAICAR. ADE2 is transcribed from a 558 bp intergenic promoter region, which is bound by NRF-1 and Sp1 at sites within the 215-260 region. The intergenic region is an integrated bidirectional promoter and a novel initiator-like element plays a central role in coordinating expression of the divergently transcribed ADE2 and GPAT genes. The ADE2 mRNA levels increase approximately five to six fold in G_1/S phase of the cell cycle over those in G_0 phase in synchronized rat 3Y1 fibroblasts. The ADE2 gene encodes enzyme of AIRC at step 6 and SAICR synthetase at step 7 in *de novo* purine nucleotide synthesis. The ADE2 encoded enzyme has no ATP dependence and no common cofactors or metals are required for catalysis. Activities of ADE2 enzyme are found in lysates of human erythrocytes, thrombocytes and leukocytes and in homogenate of the stomach biopsy sample, but not in blood plasma and bile.

REFERENCES

1. Chen, Z.D., et al. 1990. Cloning of a chicken liver cDNA encoding 5-aminoimidazole ribonucleotide carboxylase and 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide synthetase by functional complementation of *Escherichia coli* pur mutants. Proc. Natl. Acad. Sci. USA 87: 3097-3101.
2. Alenin, V.V., et al. 1990. Testing the activity of enzymes responsible for biosynthesis of purine nucleotides AIR-carboxylase and SAICAR-synthase in human cell extracts. Vopr. Med. Khim. 36: 59-63.
3. Firestine, S.M. and Davisson V.J. 1994. Carboxylases in *de novo* purine biosynthesis. Characterization of the Gallus gallus bifunctional enzyme. Biochemistry 33: 11917-11926.
4. Firestine, S.M., et al. 1994. Reactions catalyzed by 5-aminoimidazole ribonucleotide carboxylases from *Escherichia coli* and *Gallus gallus:* a case for divergent catalytic mechanisms. Biochemistry 33: 11927-11934.

CHROMOSOMAL LOCATION

Genetic locus: PAICS (human) mapping to 4q12.

PRODUCT

ADE2 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 ADE2 shRNA Plasmid (h): sc-105042-SH and ADE2 shRNA (h) Lentiviral Particles: sc-105042-V as alternate gene silencing products.

For independent verification of ADE2 (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-105042A, sc-105042B and sc-105042C.

RESEARCH USE

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

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

ADE2 siRNA (h) is recommended for the inhibition of ADE2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ADE2 gene expression knockdown using RT-PCR Primer: ADE2 (h)-PR: sc-105042-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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