

ACYP1 siRNA (h): sc-92173

BACKGROUND

Acylphosphatase is a cytosolic enzyme that catalyzes the hydrolysis of the carboxyl-phosphate bond of acylphosphates. Two acylphosphatase isoenzymes exist: ACYP1, also known as erythrocyte acylphosphatase, and ACYP2, also known as muscle acylphosphatase. The two isoenzymes share 60% homology and have the same substrate specificity, although ACYP1 has higher catalytic activity than ACYP2. ACYP2 has emerged as a significant model system to study protein misfolding and aggregation. It is particularly suitable for these studies because ACYP2 is a small, simple protein of only 98 amino acids consisting of a five-stranded antiparallel β -sheet and two parallel α -helices. Mutations in ACYP2 between residues 16-31 and 87-98, which includes its phosphate binding site at Arg-23, significantly increase the rate of aggregation. These mutations correlate with changes in the hydrophobicity of ACYP2 and a conversion of the α -helical structures to β -sheets. Therefore, a reduction in the net charge of a protein may be a key determinant in some forms of protein deposition diseases.

REFERENCES

1. Liguri, G., Camici, G., Manao, G., Cappugi, G., Nassi, P., Modesti, A. and Ramponi, G. 1986. A new acylphosphatase isoenzyme from human erythrocytes: purification, characterization, and primary structure. *Biochemistry* 25: 8089-8094.
2. Degl'Innocenti, D., Berti, A., Stefani, M., Liguri, G. and Ramponi, G. 1990. Immunoaffinity purification and immunoassay determination of human erythrocyte acylphosphatase. *Biotechnol. Appl. Biochem.* 12: 450-459.
3. Nassi, P., Nediani, C., Liguri, G., Taddei, N. and Ramponi, G. 1991. Effects of acylphosphatase on the activity of erythrocyte membrane Ca^{2+} pump. *J. Biol. Chem.* 266: 10867-10871.
4. Fiaschi, T., Raugei, G., Marzocchini, R., Chiarugi, P., Cirri, P. and Ramponi, G. 1995. Cloning and expression of the cDNA coding for the erythrocyte isoenzyme of human acylphosphatase. *FEBS Lett.* 367: 145-148.
5. Fiaschi, T., Marzella, R., Veggli, D., Marzocchini, R., Raugei, G., Rocchi, M. and Ramponi, G. 1998. Assignment of the human erythrocyte acylphosphatase gene (ACYP1) to chromosome band 14q24.3. *Cytogenet. Cell Genet.* 81: 235-236.

CHROMOSOMAL LOCATION

Genetic locus: ACYP1 (human) mapping to 14q24.3.

PRODUCT

ACYP1 siRNA (h) is a pool of 2 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 ACYP1 shRNA Plasmid (h): sc-92173-SH and ACYP1 shRNA (h) Lentiviral Particles: sc-92173-V as alternate gene silencing products.

For independent verification of ACYP1 (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-92173A and sc-92173B.

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

ACYP1 siRNA (h) is recommended for the inhibition of ACYP1 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

ACYP1 (2-RE16): sc-134246 is recommended as a control antibody for monitoring of ACYP1 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 ACYP1 gene expression knockdown using RT-PCR Primer: ACYP1 (h)-PR: sc-92173-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.