SANTA CRUZ BIOTECHNOLOGY, INC.

LRAT siRNA (m): sc-60965

BACKGROUND

Lecithin retinol acyltransferase (LRAT) is a membrane bound enzyme that catalyzes the transfer of an acyl group from the sn-1 position of lecithin to vitamin A, which generates all-*trans*-retinyl esters (tREs) in the liver, some extrahepatic tissues (such as the lung), and retinal pigmented epithelium. LRAT can also exchange palmitoyl groups between RPE65, a tRE binding protein essential for vision, and tREs, which is important for the operation of the visual pathway. LRAT is essential for the dietary mobilization, transport and storage of vitamin A, as well as the synthesis of the visual pigment chromophore. LRAT monomers interact in membranes to form homodimers through disulfide bond formation. A loss of LRAT correlates with an early onset severe retinal dystrophy and severe retinyl ester deprivation, while a reduction in LRAT expression may be associated with invasive bladder cancer.

REFERENCES

1. Jurukovski, V. and Simon, M. 1999. Reduced lecithin:retinol acyl transferase activity in cultured squamous cell carcinoma lines results in increased substrate-driven retinoic acid synthesis. Biochim. Biophys. Acta 1436: 479-490.
2. Mondal, M.S., Ruiz, A., Hu, J., Bok, D. and Rando, R.R. 2001. Two histidine residues are essential for catalysis β transferase. FEBS Lett. 489: 14-18.
3. Fishkin, N., Yefidoff, R., Gollipalli, D.R. and Rando, R.R. 2005. On the mechanism of isomerization of all-*trans*-retinol esters to 11-*cis*-retinol in retinal pigment epithelial cells: 11-fluoro-all-*trans*-retinol as substrate/inhibitor in the visual cycle. Bioorg. Med. Chem. 13: 5189-5194.
4. Harrison, E.H. 2005. Mechanisms of digestion and absorption of dietary vitamin A. Annu. Rev. Nutr. 25: 87-103.
5. Trevino, S.G., Schuschereba, S.T., Bowman, P.D. and Tsin, A. 2005. Lecithin:retinol acyltransferase in ARPE-19. Exp. Eye Res. 80: 897-900.
6. Kaschula, C.H., Jin, M.H., Desmond-Smith, N.S. and Travis, G.H. 2005. Acyl CoA:retinol acyltransferase (ARAT) activity is present in bovine retinal pigment epithelium. Exp. Eye Res. 82: 111-112.
7. Mata, N.L., Ruiz, A., Radu, R.A., Bui, T.V. and Travis, G.H. 2005. Chicken retinas contain a retinoid isomerase activity that catalyzes the direct conversion of all-*trans*-retinol to 11-*cis*-retinol. Biochemistry 44: 11715-11721.

CHROMOSOMAL LOCATION

Genetic locus: Lrat (mouse) mapping to 3 E3.

PRODUCT

LRAT siRNA (m) 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 LRAT shRNA Plasmid (m): sc-60965-SH and LRAT shRNA (m) Lentiviral Particles: sc-60965-V as alternate gene silencing products.

For independent verification of LRAT (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60965A, sc-60965B and sc-60965C.

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

LRAT siRNA (m) is recommended for the inhibition of LRAT expression in mouse 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

LRAT (M34-P1F10): sc-101391 is recommended as a control antibody for monitoring of LRAT 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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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 LRAT gene expression knockdown using RT-PCR Primer: LRAT (m)-PR: sc-60965-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.