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LDLR siRNA (r): sc-156112

BACKGROUND

LDLR (low density lipoprotein receptor) is a member of the LDL receptor gene family, which includes LDLR, LRP, megalin, VLDLR and ApoER2. The LDL receptor family is characterized by a cluster of cysteine-rich class A repeats, epidermal growth factor (EGF)-like repeats, YWTD repeats and an O-linked sugar domain. The LDL receptor is a cell surface transmembrane protein that mediates the uptake of low density lipoprotein and its degradation in the lysosome, which provides cholesterol to cells. The cytoplasmic domain of the LDL receptor is necessary for the receptor to cluster in coated pits, which promotes the rapid endocytosis of bound LDL. Mutations in LDLR cause the autosomal dominant disease, familial hypercholesterolemia (FH), which promotes premature coronary atherosclerosis.

REFERENCES

1. Davis, C.G., et al. 1986. The J.D. mutation in familial hypercholesterolemia: amino acid substitution in cytoplasmic domain impedes internalization of LDL receptors. *Cell* 45: 15-24.
2. Davis, C.G., et al. 1987. The low density lipoprotein receptor. Identification of amino acids in cytoplasmic domain required for rapid endocytosis. *J. Biol. Chem.* 262: 4075-4082.
3. Hobbs, H.H., et al. 1992. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum. Mutat.* 1: 445-466.
4. Fass, D., et al. 1997. Molecular basis of familial hypercholesterolemia from structure of LDL receptor module. *Nature* 388: 691-693.
5. Day, I.N., et al. 1997. Spectrum of LDL receptor gene mutations in heterozygous familial hypercholesterolemia. *Hum. Mutat.* 10: 116-127.
6. Trommsdorff, M., et al. 1999. Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97: 689-701.

CHROMOSOMAL LOCATION

Genetic locus: *Ldlr* (rat) mapping to 8q13.

PRODUCT

LDLR siRNA (r) 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 LDLR shRNA Plasmid (r): sc-156112-SH and LDLR shRNA (r) Lentiviral Particles: sc-156112-V as alternate gene silencing products.

For independent verification of LDLR (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156112A, sc-156112B and sc-156112C.

PROTOCOLS

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

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

LDLR siRNA (r) is recommended for the inhibition of LDLR expression in rat 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

LDLR (C7): sc-18823 is recommended as a control antibody for monitoring of LDLR 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 LDLR gene expression knockdown using RT-PCR Primer: LDLR (r)-PR: sc-156112-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.