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SANTA CRUZ BIOTECHNOLOGY, INC.

RAP siRNA (m): sc-152700

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Members of the LDL receptor gene family, including LDLR (low density lipoprotein receptor), LRP (low density lipoprotein related protein), Megalin (also designated GP330), VLDLR (very low density lipoprotein receptor) and ApoER2, are characterized by a cluster of cysteine-rich class A repeats, epidermal growth factor (EGF)-like repeats, YWTD repeats and an O-linked sugar domain. LRP, also designated α-2-Macroglobulin receptor, is an endocytic receptor that mediates the uptake of at least 15 ligands, including α-2-Macroglobulin and apoE. LRP is cleaved into a membrane subunit and an extracellular subunit, which remain non-covalently associated. Proper folding and trafficking of LRP is facilitated by the receptor-associated protein (RAP), a molecular chaperone. The uptake of all known ligands through LRP can be blocked by RAP, which induces a conformational change in the receptor that renders it unable to bind ligands. LRP, which is expressed in brain, liver and lung, is also implicated in Alzheimer's disease (AD), as the human LRP gene localizes to a potential AD locus on chromosome 12.

REFERENCES

1. Vash, B., et al. 1998. Three complement-type repeats of the low-density lipoprotein receptor-related protein define a common binding site for RAP, PAI-1 and lactoferrin. Blood 92: 3277-3285.
2. 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.
3. Mikhailenko, I., et al. 1999. Functional domains of the very low density lipoprotein receptor: molecular analysis of ligand binding and acid-dependent ligand dissociation mechanisms. J. Cell Sci. 112: 3269-3281.
4. Lambert, J.C., et al. 1999. Is the LDL receptor-related protein involved in Alzheimer's disease? Neurogenetics 2: 109-113.
5. Neels, J.G., et al. 1999. The second and fourth cluster of class A cysteine-rich repeats of the low density lipoprotein receptor-related protein share ligand-binding properties. J. Biol. Chem. 274: 31305-31311.
6. Kang, D.E., et al. 2000. Modulation of Amyloid β-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. J. Clin. Invest. 106: 1159-1166.

CHROMOSOMAL LOCATION

Genetic locus: Lrpap1 (mouse) mapping to 5 B2.

PRODUCT

RAP 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 RAP shRNA Plasmid (m): sc-152700-SH and RAP shRNA (m) Lentiviral Particles: sc-152700-V as alternate gene silencing products.

For independent verification of RAP (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-152700A, sc-152700B and sc-152700C.

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

RAP siRNA (m) is recommended for the inhibition of RAP 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

RAP (E-7): sc-515625 is recommended as a control antibody for monitoring of RAP 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RAP gene expression knockdown using RT-PCR Primer: RAP (m)-PR: sc-152700-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.