

Clusterin siRNA (r): sc-270340

BACKGROUND

Clusterin, also designated complement lysis inhibitor (CLI), apolipoprotein J (ApoJ), sulfated glycoprotein 2 (SGP2), SP-40 and testosterone-repressed prostate message 2 (TRPM2), is a secretory, heterodimeric glycoprotein that influences immune regulation, cell adhesion, transformation, lipid transportation, tissue remodeling, membrane recycling and cell-cell interactions. Clusterin is synthesized as a 449 amino acid polypeptide that is post-translationally cleaved at an internal bond between Arg 227 and Ser 228. Two subunits, α and β , are associated through disulfide bonds. The β subunit (also called ApoJ α) corresponds to residues 23-227. The α subunit (also called ApoJ β) corresponds to residues 228-449. Overexpression of Clusterin appears to be more common in late stages of mammary tumor progression. Clusterin markedly influences β -Amyloid structure and neuritic toxicity *in vivo* and may influence Alzheimer's disease pathogenesis.

REFERENCES

1. de Silva, H.V., et al. 1990. Apolipoprotein J: structure and tissue distribution. *Biochemistry* 29: 5380-5389.
2. Rosenberg, M.E., et al. 2002. Apolipoprotein J/Clusterin prevents a progressive glomerulopathy of aging. *Mol. Cell. Biol.* 22: 1893-1902.
3. Chen, X., et al. 2003. Clusterin as a biomarker in murine and human intestinal neoplasia. *Proc. Natl. Acad. Sci. USA* 100: 9530-9535.
4. Leskov, K.S., et al. 2003. Synthesis and functional analyses of nuclear Clusterin, a cell death protein. *J. Biol. Chem.* 278: 11590-11600.
5. Gwon, J.S., et al. 2004. Expression of Clusterin in Muller cells of the rat retina after pressure-induced ischemia. *Glia* 47: 35-45.
6. Pucci, S., et al. 2004. Modulation of different Clusterin isoforms in human colon tumorigenesis. *Oncogene* 23: 2298-2304.
7. Criswell, T., et al. 2005. Delayed activation of Insulin-like growth factor-1 receptor/Src/MAPK/Egr-1 signaling regulates clusterin expression, a pro-survival factor. *J. Biol. Chem.* 280: 14212-14221.
8. Xie, D., et al. 2005. Up-regulated expression of cytoplasmic Clusterin in human ovarian carcinoma. *Cancer* 103: 277-283.

CHROMOSOMAL LOCATION

Genetic locus: Clu (rat) mapping to 15p12.

PRODUCT

Clusterin siRNA (r) is a target-specific 19-25 nt siRNA 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 Clusterin shRNA Plasmid (r): sc-270340-SH and Clusterin shRNA (r) Lentiviral Particles: sc-270340-V as alternate gene silencing products.

For independent verification of Clusterin (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-270340A and sc-270340B.

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

Clusterin siRNA (r) is recommended for the inhibition of Clusterin 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

Clusterin- α (B-5): sc-5289 is recommended as a control antibody for monitoring of Clusterin 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 Clusterin gene expression knockdown using RT-PCR Primer: Clusterin (r)-PR: sc-270340-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.

PROTOCOLS

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