



Clathrin LCA siRNA (m): sc-35069

BACKGROUND

Clathrin is a major cytosolic coat protein in pits and vesicles originating from the plasma membrane and the *trans*-Golgi network. In receptor-mediated endocytosis, receptor proteins are engulfed by clathrin-coated vesicles. Clathrin is composed of three heavy chains and three light chains which associate non-covalently to form a triskelion structure. Clathrin light chain regulates the self-assembly of triskelions onto intracellular membranes. Clathrin light chain subunits (LCA and LCB) contribute to regulation of coated vesicle formation to sort proteins during receptor-mediated endocytosis and organelle biogenesis. Although LCA and LCB are encoded by two discrete genes sharing only 60% homology, they have certain features in common. Both LCA and LCB undergo alternative mRNA splicing, which results in the generation of tissue-specific isoforms. Additionally, in the brain, LCA and LCB contain inserted sequences that form higher molecular weight isoforms. These sequences insert at similar cytoplasmic domain encoding regions for both LCA and LCB.

REFERENCES

1. Pearse, B.M. 1987. Clathrin and coated vesicles. *EMBO J.* 6: 2507-2512.
2. Pearse, B.M. and Crowther, R.A. 1987. Structure and assembly of coated vesicles. *Annu. Rev. Biophys. Biochem.* 16: 49-68.
3. Jackson, A.P., et al. 1988. Structure of human Clathrin light chains. Conservation of light chain polymorphism in three mammalian species. *J. Biol. Chem.* 263: 16688-16695.
4. Acton, S.L., et al. 1990. Predominance of Clathrin light chain LCB correlates with the presence of a regulated secretory pathway. *J. Cell Biol.* 111: 1419-1426.
5. Wong, D.H., et al. 1990. Neuron-specific expression of high-molecular-weight Clathrin light chain. *J. Neurosci.* 10: 3025-3031.
6. Acton, S.L., et al. 1993. Alteration of Clathrin light chain expression by transfection and gene disruption. *Mol. Biol. Cell* 4: 647-660.

CHROMOSOMAL LOCATION

Genetic locus: Clta (mouse) mapping to 4 B1.

PRODUCT

Clathrin LCA 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Clathrin LCA shRNA Plasmid (m): sc-35069-SH and Clathrin LCA shRNA (m) Lentiviral Particles: sc-35069-V as alternate gene silencing products.

For independent verification of Clathrin LCA (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-35069A, sc-35069B and sc-35069C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Clathrin LCA shRNA Plasmid (m) is recommended for the inhibition of Clathrin LCA 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 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

Clathrin LC (CON.1): sc-12735 is recommended as a control antibody for monitoring of Clathrin LCA 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 Clathrin LCA gene expression knockdown using RT-PCR Primer: Clathrin LCA (m)-PR: sc-35069-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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