

COPE siRNA (h): sc-41198

BACKGROUND

Membrane and vesicular trafficking in the early secretory pathway are mediated by non-Clathrin COP (coat protein) I-coated vesicles. COPI-coated vesicles mediate retrograde transport from the Golgi back to the ER and intra-Golgi transport. The cytosolic precursor of the COPI coat, the heptameric coatomer complex, is composed of two subcomplexes. The first consists of the COB, COPG, COPD and COPZ subunits (also known as β -, γ -, δ - and ζ -COP, respectively), which are distantly homologous to AP Clathrin adaptor subunits. The second consists of the COPA, β' -COP and COPE subunits (also known as α -COP, COPP and ϵ -COP, respectively).

REFERENCES

1. Lowe, M. and Kreis, T.E. 1995. *In vitro* assembly and disassembly of coatomer. J. Biol. Chem. 270: 31364-31371.
2. Daro, E., et al. 1997. Inhibition of endosome function in CHO cells bearing a temperature-sensitive defect in the coatomer (COPI) component ϵ -COP. J. Cell Biol. 139: 1747-1759.
3. Duden, R., et al. 1998. ϵ -COP is a structural component of coatomer that functions to stabilize α -COP. EMBO J. 17: 985-995.
4. Harter, C. and Wieland, F.T. 1998. A single binding site for dilysine retrieval motifs and p23 within the γ subunit of coatomer. Proc. Natl. Acad. Sci. USA 95: 11649-11654.
5. Andersson, H., et al. 1999. Protein targeting to endoplasmic reticulum by dilysine signals involves direct retention in addition to retrieval. J. Biol. Chem. 274: 15080-15084.
6. Chow, C.W., et al. 1999. The epithelial Na^+/H^+ exchanger, NHE3, is internalized through a Clathrin-mediated pathway. J. Biol. Chem. 274: 37551-37558.
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CHROMOSOMAL LOCATION

Genetic locus: COPE (human) mapping to 19p13.11.

PRODUCT

COPE siRNA (h) is a pool of 2 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 COPE shRNA Plasmid (h): sc-41198-SH and COPE shRNA (h) Lentiviral Particles: sc-41198-V as alternate gene silencing products.

For independent verification of COPE (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41198A and sc-41198B.

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

COPE siRNA (h) is recommended for the inhibition of COPE expression in human 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

COPE (A-4): sc-133195 is recommended as a control antibody for monitoring of COPE 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 MarkerTM 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 COPE gene expression knockdown using RT-PCR Primer: COPE (h)-PR: sc-41198-PR (20 μl). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.