SANTA CRUZ BIOTECHNOLOGY, INC.

NECAP 1 siRNA (h): sc-95850

BACKGROUND

Clathrin-coated vesicles (CCVs) select cargo for endocytotic entry into cells and generate carrier vesicles for transport between the endosomal system and *trans*-Golgi network (TGN). NECAP 1 and NECAP 2 are essential protein paralogues for clathrin-mediated membrane trafficking that are enriched in CCV coats. NECAP 1 (NECAP endocytosis associated 1), also known as adaptin ear-binding coat-associated protein 1, is a 275 amino acid cell membrane protein belonging to the NECAP family that localizes to the clathrin-coated vesicle membrane. NECAP 1 is encoded by a gene that maps to human chromosome 12p13.31 and contains two WXXF motifs that are used to mediate binding of accessory proteins to ear domains of various proteins such as AP-1, AP-2 and GGAs. Expressed primarily in brain and existing as two alternatively spliced isoforms, NECAP 1 colocalizes with AP-2 at the plasma membrane by binding AP-2s α-ear domain.

REFERENCES

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2. Ritter, B., Philie, J., Girard, M., Tung, E.C., Blondeau, F. and McPherson, P.S. 2003. Identification of a family of endocytic proteins that define a new α-adaptin ear-binding motif. EMBO Rep. 4: 1089-1095.
3. Ritter, B., Blondeau, F., Denisov, A.Y., Gehring, K. and McPherson, P.S. 2004. Molecular mechanisms in clathrin-mediated membrane budding revealed through subcellular proteomics. Biochem. Soc. Trans. 32: 769-773.
4. Mattera, R., Ritter, B., Sidhu, S.S., McPherson, P.S. and Bonifacino, J.S. 2004. Definition of the consensus motif recognized by γ-adaptin ear domains. J. Biol. Chem. 279: 8018-8028.
5. McPherson, P.S. and Ritter, B. 2005. Peptide motifs: building the clathrin machinery. Mol. Neurobiol. 32: 73-87.

CHROMOSOMAL LOCATION

Genetic locus: NECAP1 (human) mapping to 12p13.31.

PRODUCT

NECAP 1 siRNA (h) 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 NECAP 1 shRNA Plasmid (h): sc-95850-SH and NECAP 1 shRNA (h) Lentiviral Particles: sc-95850-V as alternate gene silencing products.

For independent verification of NECAP 1 (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-95850A, sc-95850B and sc-95850C.

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 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

NECAP 1 siRNA (h) is recommended for the inhibition of NECAP 1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NECAP 1 gene expression knockdown using RT-PCR Primer: NECAP 1 (h)-PR: sc-95850-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.