SANTA CRUZ BIOTECHNOLOGY, INC.

epsin 1 siRNA (h): sc-35323

BACKGROUND

Epsin 1 (EPN1) is an endocytic accessory protein, with significant similarity to the *Xenopus* mitotic phosphoprotein MP90. Epsin 1 interacts with Eps15 (the α subunit of the Clathrin adaptor AP2), Clathrin and other accessory proteins. The mitotic phosphorylation of these proteins may be one of the mechanisms by which the invagination of Clathrin-coated pits is blocked in mitosis. Both epsin and Eps15, like other cytosolic components of the synaptic vesicle endocytic machinery, undergo constitutive phosphorylation and depolarization-dependent dephosphorylation in nerve terminals. Epsin 1 also contributes to the mechanism of Clathrin-vesicle-dependent endocytosis. The human epsin 1 protein contains an epsin N-terminal homology (ENTH) region and a single Clathrin-binding (LVDLD) motif. Epsin 1 localizes to the leading edge of a vesicular coated pit where the membrane is being actively bent.

REFERENCES

1. Chen, H., et al. 1998. Epsin is an EH-domain-binding protein implicated in Clathrin-mediated endocytosis. Nature 394: 793-797.
2. Rosenthal, et al. 1999. The epsins define a family of proteins that interact with components of the Clathrin coat and contain a new protein module. J. Biol. Chem. 274: 33959-33965.
3. Morinaka, K., et al. 1999. Epsin binds to the EH domain of POB1 and regulates receptor-mediated endocytosis. Oncogene 18: 5915-5922.
4. Drake, M.T., et al. 2000. Epsin binds to Clathrin by associating directly with the Clathrin-terminal domain. Evidence for cooperative binding through two discrete sites. J. Biol. Chem. 275: 6479-6489.
5. Oldham, C.E., et al. 2002. The ubiquitin-interacting motifs target the endocytic adaptor protein epsin for ubiquitination. Curr. Biol. 12: 1112-1116.
6. Ford, M.G., et al. 2002. Curvature of Clathrin-coated pits driven by epsin. Nature 419: 361-366.
7. Wendland, B. 2002. Epsins: adaptors in endocytosis? Nat. Rev. Mol. Cell Biol. 3: 971-977.
8. Hussain, N.K., et al. 2003. A role for epsin N-terminal homology/AP180 N-terminal homology (ENTH/ANTH) domains in tubulin binding. J. Biol. Chem. 278: 28823-28830.

CHROMOSOMAL LOCATION

Genetic locus: EPN1 (human) mapping to 19q13.42.

PRODUCT

epsin 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 epsin 1 shRNA Plasmid (h): sc-35323-SH and epsin 1 shRNA (h) Lentiviral Particles: sc-35323-V as alternate gene silencing products.

For independent verification of epsin 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-35323A, sc-35323B and sc-35323C.

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

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

GENE EXPRESSION MONITORING

epsin 1 (C-11): sc-55556 is recommended as a control antibody for monitoring of epsin 1 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 epsin 1 gene expression knockdown using RT-PCR Primer: epsin 1 (h)-PR: sc-35323-PR (20 µl, 521 bp). 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.