

epsin 2 siRNA (h): sc-40511

BACKGROUND

Elucidation of the mechanism by which receptor tyrosine kinases (RTKs) modulate cellular physiology in response to stimuli is critical to the understanding of growth regulation. Miscues in RTK signaling pathways can result in cellular transformation and ultimately in cancer. Two novel EGF receptor substrates designated EGF-receptor pathway substrates 8 and 15, or Eps8 and Eps15, have been described. Epsin is a 90 kDa binding partner to Eps15. Both epsin and Eps15 have an ubiquitous tissue distribution but are concentrated in presynaptic nerve terminals specialized for the Clathrin-mediated endocytosis of synaptic vesicles. Disruption of epsin function blocks Clathrin-mediated endocytosis. Epsin along with its binding partner Eps15 is proposed to be involved in the assistance of Clathrin coat rearrangement during Clathrin coated pit invagination. Epsin 2 and epsin 2a are also associated with Clathrin-mediated endocytosis and are enriched in the brain in the peri-Golgi region.

REFERENCES

1. Reynolds, F.H., Jr., et al. 1981. Human transforming growth factors induces tyrosine phosphorylation of EGF receptors. *Nature* 292: 259-262.
2. Ciardiello, F., et al. 1991. Differential expression of epidermal growth factor-related proteins in human colorectal tumors. *Proc. Natl. Acad. Sci. USA* 88: 7792-7796.
3. Fazioli, F., et al. 1993. Eps8, a substrate for the epidermal growth factor receptor kinase, enhances EGF-dependent mitogenic signals. *EMBO J.* 12: 3799-3808.
4. Fazioli, F., et al. 1993. Eps15, a novel tyrosine kinase substrate, exhibits transforming activity. *Mol. Cell. Biol.* 13: 5814-5828.
5. Chen, H., et al. 1998. Epsin is an EH-domain-binding protein implicated in Clathrin-mediated endocytosis. *Nature* 394: 793-797.
6. Sengar, A.S., et al. 1999. The EH and SH3 domain ESE proteins regulate endocytosis by linking to dynamin and Eps15. *EMBO J.* 18: 1159-1171.
7. Chen, H., et al. 1999. The interaction of epsin and Eps15 with the Clathrin adaptor AP-2 is inhibited by mitotic phosphorylation and enhanced by stimulation-dependent dephosphorylation in nerve terminals. *J. Biol. Chem.* 274: 3257-3260.

CHROMOSOMAL LOCATION

Genetic locus: EPN2 (human) mapping to 17p11.2.

PRODUCT

epsin 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see epsin 2 shRNA Plasmid (h): sc-40511-SH and epsin 2 shRNA (h) Lentiviral Particles: sc-40511-V as alternate gene silencing products.

For independent verification of epsin 2 (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-40511A, sc-40511B and sc-40511C.

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

epsin 2 siRNA (h) is recommended for the inhibition of epsin 2 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 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

epsin 2 (F-10): sc-376788 is recommended as a control antibody for monitoring of epsin 2 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 epsin 2 gene expression knockdown using RT-PCR Primer: epsin 2 (h)-PR: sc-40511-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.