

Eps8 siRNA (m): sc-40504

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. Eps8 and Eps15 become tyrosine phosphorylated subsequent to EGF stimulation. Overexpression of Eps15 in NIH/3T3 cells causes cellular transformation, implying involvement in the regulation of cell proliferation. Eps15 is capable of binding the amino-terminal portion of Crk via a conserved proline-rich domain, characteristic of all Crk binding proteins. Overexpression of Eps8 in both fibroblasts and hematopoietic cells results in an increased mitogenic response to EGF. Eps8 has been shown to associate with the EGF receptor despite its lack of a functional SH2 domain. Further characterization suggests the protein has both a PH domain and a SH3 domain, the functional significance of which are not yet known.

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. Wong, W.T., et al. 1994. Evolutionary conservation of the EPS8 gene and its mapping to human chromosome 12q23-q24. *Oncogene* 9: 3057-3061.
6. Schumacher, C., et al. 1995. The SH3 domain of Crk binds specifically to a conserved proline-rich motif in Eps15 and Eps15R. *J. Biol. Chem.* 270: 15341-15347.

CHROMOSOMAL LOCATION

Genetic locus: Eps8 (mouse) mapping to 6 G1.

PRODUCT

Eps8 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 Eps8 shRNA Plasmid (m): sc-40504-SH and Eps8 shRNA (m) Lentiviral Particles: sc-40504-V as alternate gene silencing products.

For independent verification of Eps8 (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-40504A, sc-40504B and sc-40504C.

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

Eps8 siRNA (m) is recommended for the inhibition of Eps8 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

Eps8 (F-8): sc-390257 is recommended as a control antibody for monitoring of Eps8 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 Eps8 gene expression knockdown using RT-PCR Primer: Eps8 (m)-PR: sc-40504-PR (20 μ l, 577 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.