SANTA CRUZ BIOTECHNOLOGY, INC.

Eps15R siRNA (h): sc-40507

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, as miscues in RTK signaling can result in cellular transformation events that may ultimately lead to cancer. Eps15 (EGF-receptor pathway substrate 15) is an EGF receptor substrate that become become tyrosine phosphorylated subsequent to EGF stimulation. Over expression of Eps15 in NIH/3T3 cells causes cellular transformation, implying involvement of Eps15 in the regulation of cell proliferation. Eps15R (Eps15-related), also known as Eps15L1 (epidermal growth factor receptor substrate 15-like 1), is an 864 ami-no acid protein that contains one EF-hand domain and three EH domains. Localized to a variety of places within the cell, including the nucleus, cytoplasm and membrane-coated pits, Eps15R is thought to function as a component of clathrin-coated pits. Like Eps15, Eps15R may play an important role in receptor-mediated endocytosis.

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.
7. Castagnino, P., et al. 1995. Direct binding of Eps8 to the juxtamembrane domain of EGFR is phosphotyrosine- and SH2-independent. Oncogene 10: 723-729.

CHROMOSOMAL LOCATION

Genetic locus: EPS15L1 (human) mapping to 19p13.11.

PRODUCT

Eps15R 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 Eps15R shRNA Plasmid (h): sc-40507-SH and Eps15R shRNA (h) Lentiviral Particles: sc-40507-V as alternate gene silencing products.

For independent verification of Eps15R (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-40507A, sc-40507B and sc-40507C.

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

Eps15R siRNA (h) is recommended for the inhibition of Eps15R 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 Eps15R gene expression knockdown using RT-PCR Primer: Eps15R (h)-PR: sc-40507-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.