SignalSilence® Eps15 siRNA I

#12253 Store at -20°C

☑ 10 µM in 300 µl (3 nmol)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355) orders@cellsignal.com
Support ■ 877-678-TECH (8324) info@cellsignal.com
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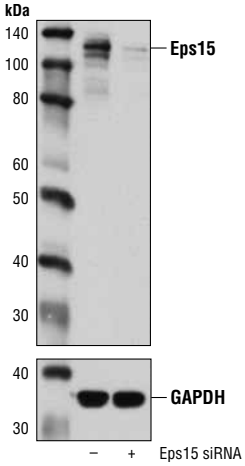
For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: SignalSilence® Eps15 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Eps15 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: Eps15 (EGFR pathway substrate 15) was originally discovered as a substrate for the kinase activity of EGFR (1). Eps15 has a tripartite structure comprising an amino terminal portion, which contains three evolutionarily conserved EH protein-protein interaction domains, a central putative coiled-coil region required for constitutive oligmerization, and a carboxy terminal domain containing multiple copies of the amino acid triplet Asp-Pro-Phe that constitute the AP2 binding domain. The carboxy terminal domain also contains two ubiquitin interaction motifs (UIMs), the last of which is indespensible for Eps15 binding to ubiquitin (1). Several lines of evidence support a role for Eps15 in clathrin-mediated endocytosis, including the endocytosis of synaptic vesicles. Eps15 binds to AP2 as well as other proteins involved in endocytosis and/or synaptic vesicle recycling, such as synaptojanin1 and epsin. Furthermore, Eps15 colocalizes with markers of the plasma membrane clathrin-coated pits and vesicles (2). Eps15 regulates the endosomal trafficking of c-Met (3) and EGFR (4), possibly by recruiting the ubiquitinated receptors to the rims of clathrin-coated pits through interaction between the ubiquitin tag and its UIMs.

The *EPS15* gene yields two isoforms that are believed to reside in distinct subcellular locations and are thus implicated in different facets of endosomal trafficking (5). Human *EPS15* has been mapped to chromosome 1p31-p32, a region displaying several nonrandom chromosomal abnormalities, including deletions in neuroblastoma and translocations in acute lymphoblastic and myeloid leukemias. Research has shown two translocations t(1;11)(p32;q11) are found in rare cases of myeloid leukemia where the Eps15 gene was fused to the HRX gene, resulting in two reciprocal fusion genes (6).



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Eps15 siRNA I (+), using Eps15 (D3K8R) Rabbit mAb #12460 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The Eps15 (D3K8R) Rabbit mAb confirms silencing of Eps15 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Eps15 siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #2060
Swiss-Prot Acc. #P42566

Storage: Eps15 siRNA I is supplied in RNAse-free water. *Aliquot and store at -20ºC.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Fazioli, F. et al. (1993) *Mol Cell Biol* 13, 5814-28.
(2) Tebar, F. et al. (1996) *J Biol Chem* 271, 28727-30.
(3) Parachoniak, C.A. and Park, M. (2009) *J Biol Chem* 284, 8382-94.
(4) Torrisi, M.R. et al. (1999) *Mol Biol Cell* 10, 417-34.
(5) Roxrud, I. et al. (2008) *J Cell Biol* 180, 1205-18.
(6) Bernard, O.A. et al. (1994) *Oncogene* 9, 1039-45.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.