SignalSilence® EGF Receptor siRNA II

Cell Signaling TECHNOLOGY®

☑ 10 µM in 300 µl
(100 Transfections)

For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #1956
Swiss-Prot Acc. #P00533

**Species Cross-Reactivity: H, (M, R)**

Description: SignalSilence® EGF Receptor siRNA from Cell Signaling Technology (CST) allows the researcher to specifically inhibit EGF Receptor 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 are rigorously tested in-house and have been shown to reduce protein expression in specified cell lines.

Background: The epidermal growth factor (EGF) receptor is a 170 kDa transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling and lysosomal degradation (1,2). Phosphorylation of EGF receptor (EGFR) at Tyr845 in the kinase domain is implicated in stabilizing the activation loop, maintaining the active state enzyme and providing a binding surface for substrate proteins (3,4). c-Src is involved in phosphorylation of EGFR at Tyr845 (5). The SH2 domain of PLCγ binds at phospho-Tyr992, resulting in activation of PLCγ-mediated downstream signaling (6). Phosphorylation of EGFR at Tyr1045 creates a major docking site for c-Cbl, an adaptor protein that leads to receptor ubiquitination and degradation following EGFR activation (7,8). The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (9). A pair of phosphorylated residues (Tyr1148 and Tyr1173) provides a docking site for the SHC scaffold protein, with both sites involved in MAP kinase signaling activation (2). Phosphorylation of EGFR at specific serine and threonine residues attenuates EGFR kinase activity. EGFR carboxy-terminal residues Ser1046 and Ser1047 are phosphorylated by CaM kinase II; mutation to either of these serines results in upregulated EGFR tyrosine autophosphorylation (10).

Directions for Use: CST recommends transfection with 100 nM EGF Receptor siRNA 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.

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.

Specificity/ Sensitivity: SignalSilence® EGF Receptor siRNA II will inhibit human, mouse and rat EGF Receptor expression.
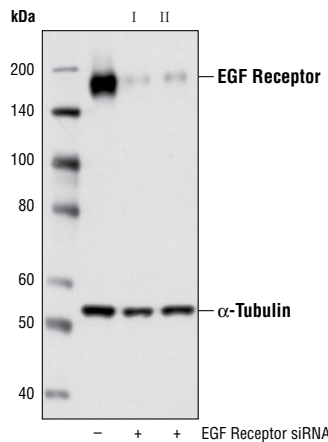
Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® EGF Receptor siRNA I #6480 (+) or SignalSilence® EGF Receptor siRNA II (+), using EGF Receptor (D38B1) XP™ Rabbit mAb #4267 and α-Tubulin (11H10) Rabbit mAb #2125. The EGF Receptor (D38B1) XP™ Rabbit mAb confirms silencing of EGF Receptor expression, while the α-Tubulin (11H10) Rabbit mAb was used to control for loading and specificity of EGF Receptor siRNA.

Storage: EGF Receptor siRNA II 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) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184–9.

(2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408–12.

(3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051–4.

(4) Hubbard, S.R. et al. (1994) *Nature* 372, 746–54.

(5) Biscardi, J.S. et al. (1999) *J Biol Chem* 274, 8335–43.

(6) Emlet, D.R. et al. (1997) *J Biol Chem* 272, 4079–86.

(7) Levkowitz, G. et al. (1999) *Mol Cell* 4, 1029–40.

(8) Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855–66.

(9) Rojas, M. et al. (1996) *J Biol Chem* 271, 27456–61.

(10) Feinmesser, R.L. et al. (1999) *J Biol Chem* 274, 16168–73.

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 **All**—all species expected   Species enclosed in parentheses are predicted to react based on 100% homology.