



ERK 5 siRNA (h): sc-35339

BACKGROUND

The activation of signal transduction pathways by growth factors, hormones and neurotransmitters is mediated through two closely related MAP kinases, p44 and p42, designated extracellular-signal related kinase 1 (ERK 1) and ERK 2, respectively. ERK proteins are regulated by dual phosphorylation at specific tyrosine and threonine sites mapping within a characteristic Thr-Glu-Tyr motif. Phosphorylation at both the Thr and Tyr residues is required for full enzymatic activation. In response to activation, MAP kinases phosphorylate downstream components on serine and threonine. Upstream MAP kinase regulators include MAP kinase kinase (MEK), MEK kinase and Raf-1. The ERK family has three additional members: ERK 3, ERK 5 and ERK 6.

REFERENCES

1. Boulton, T.G., et al. 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to Insulin and NGF. *Cell* 65: 663-675.
2. Boulton, T.G., et al. 1991. Purification and properties of ERK 1, an Insulin-stimulated MAP2 protein kinase. *Biochemistry* 30: 278-286.
3. Payne, D.M., et al. 1991. Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). *EMBO J.* 10: 885-892.
4. Haycock, J.W., et al. 1992. ERK 1 and ERK 2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at Serine 31 *in situ*. *Proc. Natl. Acad. Sci. USA* 89: 2365-2369.

CHROMOSOMAL LOCATION

Genetic locus: MAPK7 (human) mapping to 17p11.2.

PRODUCT

ERK 5 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 ERK 5 shRNA Plasmid (h): sc-35339-SH and ERK 5 shRNA (h) Lentiviral Particles: sc-35339-V as alternate gene silencing products.

For independent verification of ERK 5 (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-35339A, sc-35339B and sc-35339C.

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

ERK 5 siRNA (h) is recommended for the inhibition of ERK 5 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

ERK 5 (C-11): sc-393405 is recommended as a control antibody for monitoring of ERK 5 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 ERK 5 gene expression knockdown using RT-PCR Primer: ERK 5 (h)-PR: sc-35339-PR (20 μ l, 483 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Park, S.J., et al. 2016. BIX02189 inhibits TGF- β 1-induced lung cancer cell metastasis by directly targeting TGF- β type I receptor. *Cancer Lett.* 381: 314-322.

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.