SANTA CRUZ BIOTECHNOLOGY, INC.

eEF2K siRNA (m): sc-39012

BACKGROUND

The activity of the purified eukaryotic elongation-factor-2 kinase (eEF2K) is completely dependent on calcium and calmodulin, and autophosphorylation on serine and threonine residues is calcium/calmodulin-dependent. eEF2K is a ubiquitous protein kinase that phosphorylates and inactivates eEF2, and thus can modulate the rate of polypeptide chain elongation during translation. eEF2K is detected in skeletal muscle extracts and is phosphorylated rapidly by SAPK4, but poorly by p38, p38γ, JNK or ERK 2. SAPK4 phosphorylates eEF2K at Ser 359 and Ser 396 *in vitro,* causing its inactivation. The phosphorylation of eEF2K at Ser 359 is also induced by Insulin-like growth factor-1. Ser 359 is in close proximity to Ser 366 and the Ser 366 residue also becomes phosphorylated in response to growth factors. eEF2K is phosphorylated by p70 S6 kinase at Ser 366 and this results in the inactivation of eEF2K, especially at low (micromolar) calcium concentrations.

REFERENCES

1. Redpath, N.T. and Proud, C.G. 1993. Purification and phosphorylation of elongation factor-2 kinase from rabbit reticulocytes. Eur. J. Biochem. 212: 511-520.
2. Pavur, K.S., et al. 2000. Mapping the functional domains of elongation factor-2 kinase. Biochemistry 39: 12216-12224.
3. Knebel, A., et al. 2001. A novel method to identify protein kinase substrates: eEF2 kinase is phosphorylated and inhibited by SAPK4/p38δ. EMBO J. 20: 4360-4369.
4. Wang, X., et al. 2001. Regulation of elongation factor 2 kinase by $p90^{RSK1}$ and p70 S6 kinase. EMBO J. 20: 4370-4379.

CHROMOSOMAL LOCATION

Genetic locus: Eef2k (mouse) mapping to 7 F2.

PRODUCT

eEF2K 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see eEF2K shRNA Plasmid (m): sc-39012-SH and eEF2K shRNA (m) Lentiviral Particles: sc-39012-V as alternate gene silencing products.

For independent verification of eEF2K (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-39012A, sc-39012B and sc-39012C.

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

eEF2K siRNA (m) is recommended for the inhibition of eEF2K 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 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.

GENE EXPRESSION MONITORING

p-eEF2K (H-2): sc-377536 is recommended as a control antibody for monitoring of eEF2K 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-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 eEF2K gene expression knockdown using RT-PCR Primer: eEF2K (m)-PR: sc-39012-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Jan, A., et al. 2018. Activity of translation regulator eukaryotic elongation factor-2 kinase is increased in Parkinson disease brain and its inhibition reduces alpha synuclein toxicity. Acta Neuropathol. Commun. 6: 54.
2. Pires Da Silva, J., et al. 2020. SIRT1 protects the heart from ER stress-induced injury by promoting eEF2K/eEF2-dependent autophagy. Cells 9 pii: E426.

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.