SANTA CRUZ BIOTECHNOLOGY, INC.

p-eEF2K (C-4): sc-377562

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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.
5. Proud, C.G., et al. 2001. Interplay between Insulin and nutrients in the regulation of translation factors. Biochem. Soc. Trans. 29: 541-547.

CHROMOSOMAL LOCATION

Genetic locus: EEF2K (human) mapping to 16p12.2; Eef2k (mouse) mapping to 7 F2.

SOURCE

p-eEF2K (C-4) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 353-378 Ser 366 of eEF2K of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-377562 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

p-eEF2K (C-4) is recommended for detection of Ser 366 phosphorylated eEF2K of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-eEF2K (C-4) is also recommended for detection of correspondingly phosphorylated eEF2K in additional species, including equine and canine.

Suitable for use as control antibody for eEF2K siRNA (h): sc-39011, eEF2K siRNA (m): sc-39012, eEF2K shRNA Plasmid (h): sc-39011-SH, eEF2K shRNA Plasmid (m): sc-39012-SH, eEF2K shRNA (h) Lentiviral Particles: sc-39011-V and eEF2K shRNA (m) Lentiviral Particles: sc-39012-V.

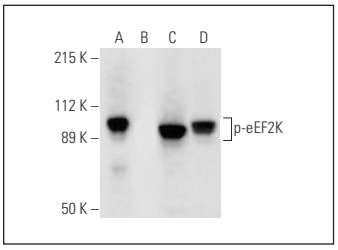
Molecular Weight of p-eEF2K: 105 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200 or MIA PaCa-2 cell lysate: sc-2285.

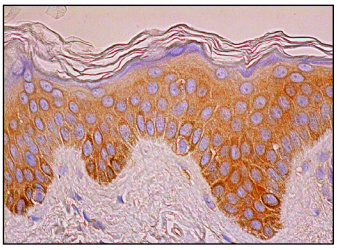
RECOMMENDED SUPPORT REAGENTS

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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) 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. 4) Immunohistochemistry: use m-IgGκ BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

DATA



Western blot analysis of eEF2K phosphorylation in untreated (**A**,**C**) and lambda protein phosphatase (sc-200312A) treated (**B**,**D**) MIA PaCa-2 whole cell lysates. Antibodies tested include p-eEF2K (C-4): sc-377562 (**A**,**B**) and eEF2K (C-12): sc-390710 (**C**,**D**).



p-eEF2K (C-4): sc-377562. Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of keratinocytes, Langerhans cells and melanocytes.

RESEARCH USE

For research use only, not for use in diagnostic procedures.