

# p-eEF2K (H-2): sc-377536

## 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, p38 $\gamma$ , 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., Petrov, A.N. and Ryazanov, A.G. 2000. Mapping the functional domains of elongation factor-2 kinase. *Biochemistry* 39: 12216-12224.

## CHROMOSOMAL LOCATION

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

## SOURCE

p-eEF2K (H-2) is a mouse monoclonal antibody specific for an epitope corresponding to a short amino acid sequence containing Ser 366 phosphorylated eEF2K of human origin.

## PRODUCT

Each vial contains 200  $\mu$ g IgG<sub>2b</sub> kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

p-eEF2K (H-2) is available conjugated to agarose (sc-377536 AC), 500  $\mu$ g/0.25 ml agarose in 1 ml, for IP; to HRP (sc-377536 HRP), 200  $\mu$ g/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-377536 PE), fluorescein (sc-377536 FITC), Alexa Fluor<sup>®</sup> 488 (sc-377536 AF488), Alexa Fluor<sup>®</sup> 546 (sc-377536 AF546), Alexa Fluor<sup>®</sup> 594 (sc-377536 AF594) or Alexa Fluor<sup>®</sup> 647 (sc-377536 AF647), 200  $\mu$ g/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor<sup>®</sup> 680 (sc-377536 AF680) or Alexa Fluor<sup>®</sup> 790 (sc-377536 AF790), 200  $\mu$ g/ml, for Near-Infrared (NIR) WB, IF and FCM.

Blocking peptide available for competition studies, sc-377536 P, (100  $\mu$ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

Alexa Fluor<sup>®</sup> is a trademark of Molecular Probes, Inc., Oregon, USA

## 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 (H-2) 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  $\mu$ g per 100-500  $\mu$ g 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 (H-2) is also recommended for detection of correspondingly phosphorylated eEF2K in additional species, including equine, canine, bovine and porcine.

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.

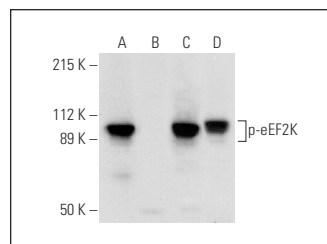
Molecular Weight of p-eEF2K: 105 kDa.

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

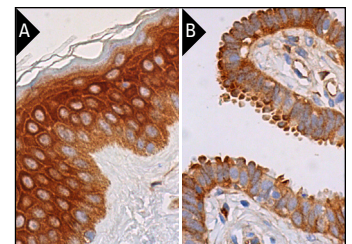
## RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> 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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850. 4) Immunohistochemistry: use m-IgG $\kappa$  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 (H-2): sc-377536 (A, B) and eEF2K (C-12): sc-390710 (C, D).



p-eEF2K (H-2): sc-377536. Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of keratinocytes, Langerhans cells and melanocytes (A). Immunoperoxidase staining of formalin fixed, paraffin-embedded human fallopian tube tissue showing cytoplasmic staining of glandular cells (B).

## RESEARCH USE

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