

p-4E-BP1/2/3 (Thr 36): sc-101624

BACKGROUND

The multisubunit eukaryotic translation initiation factor (eIF) 4F recruits 40S ribosomal subunits to the 5' end of mRNA. The eIF4F subunit eIF4E interacts directly with the mRNA 5' cap structure. Assembly of the eIF4F complex is inhibited by a family of repressor polypeptides, the eIF4E-binding proteins (4E-BPs). 4E-BP1 (also known as PHAS-1) normally binds eIF4E, inhibiting cap-dependent translation. Hyper-phosphorylation of 4E-BP1 disrupts this binding, activating cap-dependent translation. The PI 3-kinase/Akt pathway and the FRAP/mTOR kinase regulate 4E-BP1. 4E-BP1 is phosphorylated *in vivo* on multiple residues and phosphorylation by FRAP/mTOR on Threonine 37 and Threonine 46 of human 4E-BP1 may prime it for subsequent phosphorylation at sites including Serine 65 and Threonine 70. The corresponding rat residues include Threonine 36, Threonine 45, Serine 64 and Threonine 69. *In vitro*, 4E-BP1 is also phosphorylated by ataxia telangiectasia (ATM) at human Serine 112 (rat Serine 111) in response to an increase in insulin levels.

REFERENCES

1. Pause, A., et al. 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature* 371: 762-767.
2. Fadden, P., et al. 1997. Identification of phosphorylation sites in the translational regulator, PHAS-I, that are controlled by insulin and Rapamycin in rat adipocytes. *J. Biol. Chem.* 272: 10240-10247.
3. Brunn, G.J., et al. 1997. Phosphorylation of the translational repressor PHAS-I by the mammalian target of Rapamycin. *Science* 277: 99-101.
4. Gingras, A.C., et al. 1998. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt (PKB) signaling pathway. *Genes Dev.* 12: 502-513.
5. Gingras, A.C., et al. 1999. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev.* 13: 1422-1437.
6. Yang, D.Q., et al. 2000. Participation of ATM in insulin signalling through phosphorylation of eIF-4E-binding protein 1. *Nat. Cell Biol.* 2: 893-898.

SOURCE

p-4E-BP1/2/3 (Thr 36) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Thr 36 of 4E-BP1/2/3 of human origin.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

p-4E-BP1/2/3 (Thr 36) is recommended for detection of Thr 36 phosphorylated 4E-BP1/2/3 of human origin and correspondingly phosphorylated Thr 35 of mouse and rat origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1–2 µg per 100–500 µg of total protein (1 ml of cell lysate)], immunofluorescence and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

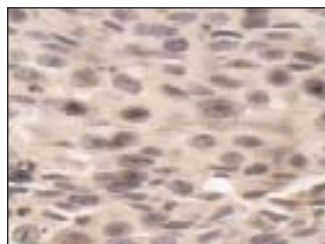
Molecular Weight of p-4E-BP1/2/3: 10-12 kDa.

Positive Controls: insulin-treated NIH/3T3 whole cell lysate, serum starved + serum NIH/3T3 whole cell lysate or human breast carcinoma tissue.

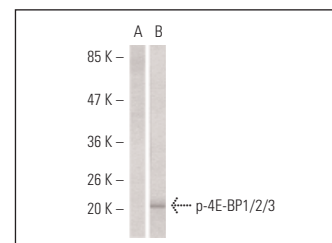
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent) 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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



p-4E-BP1/2/3 (Thr 36): sc-101624. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing nuclear and cytoplasmic localization.



p-4E-BP1/2/3 (Thr 36): sc-101624. Western blot analysis of phosphorylated 4E-BP1/2/3 expression in untreated (A) and EGF-treated (B) MDA-MB-435 whole cell lysates.

RESEARCH USE

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