

# p-4E-BP1/2/3 (Thr 45): sc-101625

## 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.

## CHROMOSOMAL LOCATION

Genetic locus: EIF4EBP1 (human) mapping to 8p12; Eif4ebp1 (mouse) mapping to 8 A2.

## SOURCE

p-4E-BP1/2/3 (Thr 45) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Thr 45 phosphorylated 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.

## APPLICATIONS

p-4E-BP1/2/3 (Thr 45) is recommended for detection of Thr 45 phosphorylated 4E-BP1/2/3 of human origin and correspondingly phosphorylated Thr 44 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: 21 kDa.

Molecular Weight of p-4E-BP2: 16 kDa.

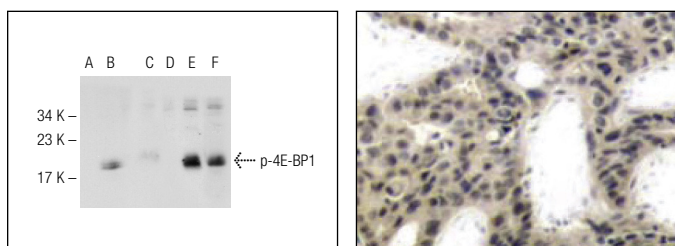
Molecular Weight of p-4E-BP3: 12 kDa.

Positive Controls: 4E-BP1 (h): 293T Lysate: sc-116590, HeLa nuclear extract: sc-2120 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), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 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



Western blot analysis of 4E-BP1 phosphorylation in non-transfected: sc-117752 (A, D), untreated human 4E-BP1 transfected: sc-116590 (B, E) and lambda protein phosphatase (sc-200312A) treated human 4E-BP1 transfected: sc-116590 (C, F) 293T whole cell lysates. Antibodies tested include p-4E-BP1/2/3 (Thr 45): sc-101625 (A, B, C) and 4E-BP1 (R-113): sc-6936 (D, E, F).

p-4E-BP1/2/3 (Thr 45): sc-101625. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing nuclear and cytoplasmic localization.

## STORAGE

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

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

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Try **p-4E-BP1/2/3 (A-10): sc-271947**, our highly recommended monoclonal alternative to p-4E-BP1/2/3 (Thr 45).