SANTA CRUZ BIOTECHNOLOGY, INC.

p-4E-BP1 (Thr 36)-R: sc-18080-R

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The Power to Question

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 PI3-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.

CHROMOSOMAL LOCATION

Genetic locus: EIF4EBP1 (human) mapping to 8p11.23; Eif4ebp1 (mouse) mapping to 8 A2.

SOURCE

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

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-18080 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

RESEARCH USE

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

APPLICATIONS

p-4E-BP1 (Thr 36)-R is recommended for detection of Thr 36 phosphorylated 4E-BP1 of mouse, rat and human 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 (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).

Suitable for use as control antibody for 4E-BP1 siRNA (h): sc-29594, 4E-BP1 siRNA (m): sc-29595, 4E-BP1 shRNA Plasmid (h): sc-29594-SH, 4E-BP1 shRNA Plasmid (m): sc-29595-SH, 4E-BP1 shRNA (h) Lentiviral Particles: sc-29594-V and 4E-BP1 shRNA (m) Lentiviral Particles: sc-29595-V.

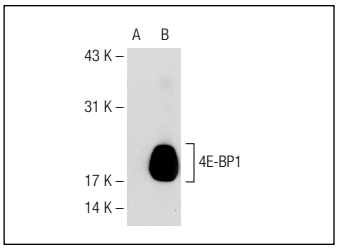
Molecular Weight of p-4E-BP1: 21 kDa.

Positive Controls: 4E-BP1 (m): 293T Lysate: sc-118030, NIH/3T3 whole cell lysate: sc-2210 or HeLa whole cell lysate: sc- 2200.

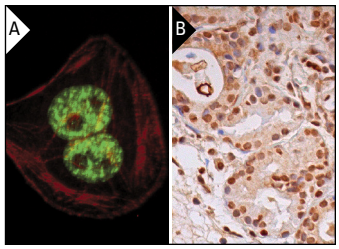
RECOMMENDED SECONDARY REAGENTS

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



p-4E-BP1 (Thr 36)-R: sc-18080-R. Western blot analysis of 4E-BP1 expression in non-transfected: sc-117752 (**A**) and mouse 4E-BP1 transfected: sc-118030 (**B**) 293T whole cell lysates.



p-4E-BP1 (Thr 36)-R: sc-18080-R. Immunofluorescence staining of methanol-fixed HeLa cells showing nuclear localization (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human salivary gland tissue showing nuclear and cytoplasmic staining of glandular cells (**B**).

STORAGE

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