4E-BP1 [pT37] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified

REF Catalog no. 700238

(See product label for lot information)

Clone/PAD: 52H37L2
Isotype: IgG
Gene ID: 1978
Protein Acc. No.: Q13541
Qty: 100 µg
Volume: 200 µl
Concentration: 0.5 mg/ml

Formulation
PBS + 0.09% azide

Immunogen
A peptide corresponding to amino acids 31-41 of Q13541.

Immunogen sequence
PGDYST[pT]PGGT

Reactivity
This antibody reacts with human 4E-BP1 [pT37]. Based on sequence identity and similarity, reactivity to mouse, rat, equine, opossum, bovine, and zebrafish is expected.

Specificity
This antibody is specific for 4E-BP1 [pT37] and does not recognize non-phosphorylated 4E-BP1.

Storage
2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Validated Applications:

	Species	Test Material	Concentration
Western Blotting	human	HeLa	4-6 µg/ml
Immunohistochemistry	human	enometrial and breast carcinoma, Hodgkin lymphoma	5-6 µg/ml
Immunofluorescence	human	HeLa	2-3 µg/ml
Flow Cytometry	human	Jurkat	0.1-0.2 µg/test
Sandwich ELISA	Detector		1-5 µg/ml

Background
Eukaryotic initiation factor 4E binding protein 1 (4E-BP1), also known as PHAS, is a ~20 kDa member of a family of eIF4E-binding proteins whose binding affinity to eIF4E is regulated by its phosphorylation. It inhibits cap-dependent translation by binding to eIF4E on the same site that overlaps the binding site for eIF4G, preventing its binding to the latter and eventually leading to an increase in mRNA translation. The phosphorylatin of 4E-BP1 is critical in determining cell fate by controlling translation initiation and apoptotic potency. 4E-BP1 is hyperphosphorylated in response to several external stimuli including hormones, growth factors, mitogens, cytokines and G-protein–coupled receptors and in response to stress conditions including nutrient deprivation. The phosphorylation of 4E-BP1 increases in response to activated phosphoinositol 3'-kinase (PI-3K) or its downstream effector Akt/PKB. 4E-BP1 is believed to mediate PI-3K and FRAP/mTOR signaling and is phosphorylated on at least six serine and threonine sites (Thr 37, Thr 46, Ser 65, Thr 70, Ser 83, and Ser 112). The phosphorylation of these sites is believed to occur in an orderly fashion where phosphorylation of threonine 37 and 46 by FRAP/mTOR is a priming step for subsequent phosphorylation of 4E-BP1 at the carboxy-terminal sites.

References

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2. Gingras, A.C., et al. (2001) Hierarchical phosphorylation of the translation inhibitor 4E-BP1.Genes Dev. 15(21):2852-2864.
3. Li, S., et al. (2002) Translational control of cell fate: availability of phosphorylation sites on translational repressor 4E-BP1 governs its proapoptotic potency. Mol. Cell Biol. 22(8):2853-2861.
4. Li, W. and B.E. Sumpio (2005) Strain-induced vascular endothelial cell proliferation requires PI3K-dependent mTOR-4E-BP1 signal pathway. Am. J. Physiol. Heart Circ. Physiol. 288(4):H1591-H1597.
5. Wang, X., et al. (2005) Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins. Mol. Cell Biol. 25(7):2558-2572.
6. Greenberg, V.L. and S.G. Zimmer (2005) Paclitaxel induces the phosphorylation of the eukaryotic translation initiation factor 4E-binding protein 1 through a Cdk1-dependent mechanism. Oncogene 24(30):4851-4860.
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8. Stephens, L., et al. (2005) Phosphoinositide 3-kinases as drug targets in cancer.Curr. Opin. Pharmacol. 5(4):357-365.
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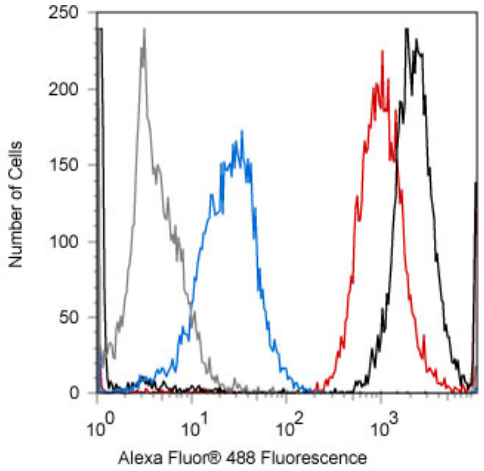
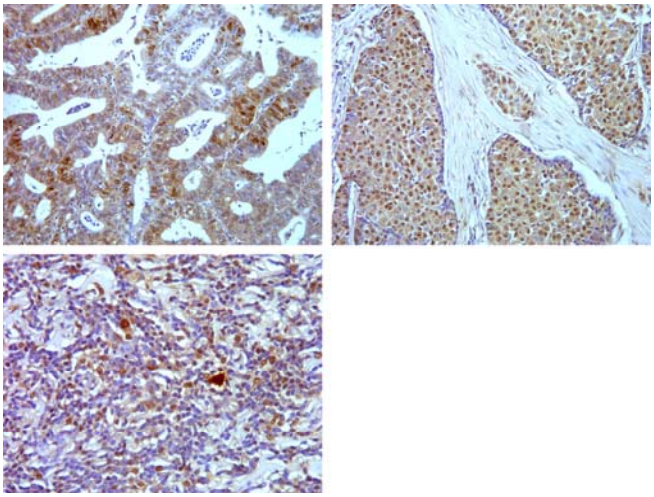


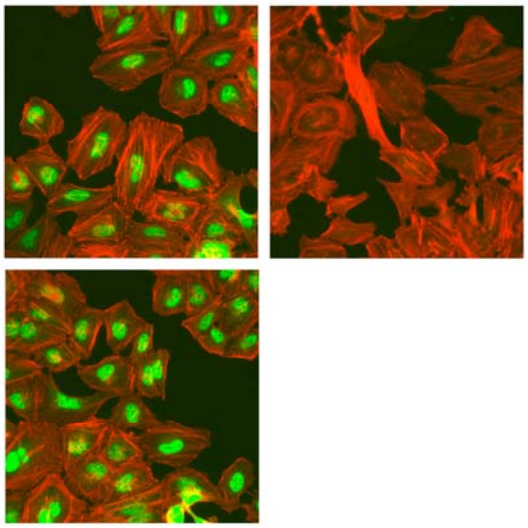
Flow cytometry of Jurkat cells labeled with rabbit anti-4E-BP1 [pT37] (Cat. No. 700238).

Jurkat cells were fixed and permeabilized using FIX & PERM® (Cat. No. GAS004) reagents. Cells were then stained with (black trace) or without (gray trace) 0.1 µg anti-4E-BP1 [pT37] followed by Alexa Fluor® 488 goat anti-rabbit Ig (Cat. No. A11008). Pre-incubation with the immunogenic phosphopeptide decreased the signal (blue trace), whereas incubation with the non-phosphopeptide did not (red trace).



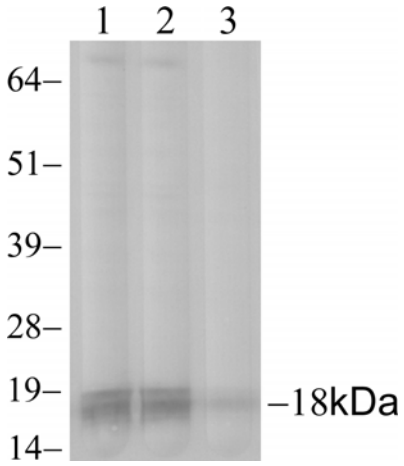
Immunohistochemistry of human endometrial and breast carcinomas and Hodgkin lymphoma tissues labeled with rabbit anti-4E-BP1 [pT37] (Cat. No. 700238).

FFPE human endomertrial carcinoma (top left), breast carcinoma (top right) and Hodgkin lymphoma (bottom) tissues were labeled with rabbit anti-4E-BP [pT37] (5 µg/ml). Tissues were detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 20x (top) or 40x (bottom) magnification. Note strong nuclear staining in tumor cells.



Immunocytochemistry of HeLa cells labeled with rabbit anti-4E-BP1 [pT37] (Cat. No. 700238).

HeLa cells labeled with rabbit anti-4E-BP1 [pT37] (2.5 µg/ml) in the absence of peptides (top left), and presence of phosphopeptide used as immunogen (top right) or non-phosphopeptide (bottom left). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody. Actin was stained with Alexa Fluor® 568 phalloidin (Cat. No. A12380).



Western blot of HeLa lysates labeled with rabbit anti-4E-BP1 [pT37] (Cat. No. 700238).

Rabbit anti-4E-BP1 [pT37] (4 µg/ml) was used to label 4E-BP1 [pT37] in HeLa lysates (lane 1). Pre-incubation of the antibody with the non-phospho peptide did not affect the signal (lane 2), while incubation with the phospho peptide resulted in loss of signal (lane 3).