

#2855 Store at -20°C

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb

- Small 100 µl (10 western blots)
- Large 300 µl (30 western blots)



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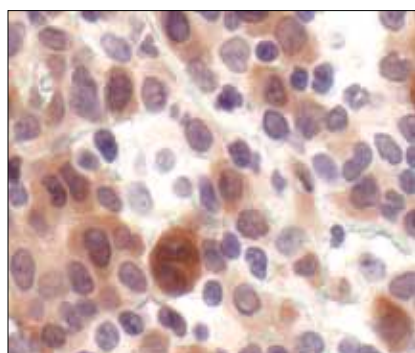
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC, F Endogenous	H, M, R, Mk, Dm	15-20 kDa	Rabbit IgG**

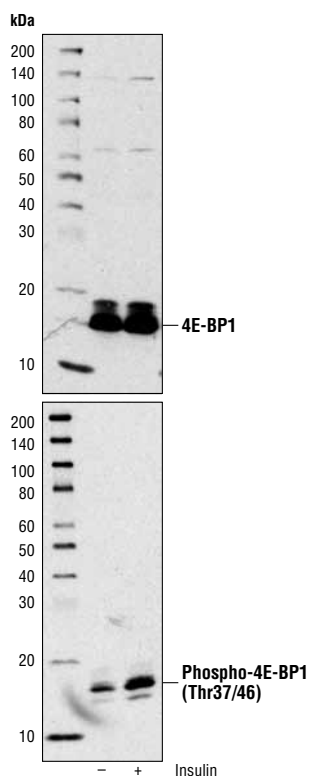
Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

Specificity/Sensitivity: Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb detects endogenous levels of 4E-BP1 only when phosphorylated at Thr37 and/or Thr46. This antibody may cross-react with 4E-BP2 and 4E-BP3 when phosphorylated at equivalent sites.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr37 and Thr46 of mouse 4E-BP1.



Immunohistochemical analysis of paraffin-embedded human lymphoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



Western blot analysis of extracts from 293T cells using 4E-BP1 Antibody #9452 (upper) and Phospho-4E-BP1 (Thr37/46) Antibody #2855 (lower). The cells were starved for 24 hours in serum-free medium and underwent a 1 hour amino acid deprivation. Amino acids were replenished for 1 hour. Cells were then either untreated (-) or treated with 100 nM insulin (+) for 30 minutes.

Entrez-Gene ID #1978
Swiss-Prot Acc. #Q13541

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:1600†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:50

For application specific protocols please see the web page for this product at www.cellsignal.com.

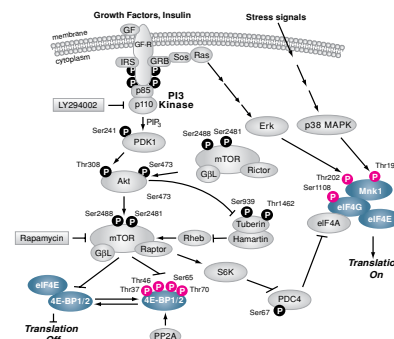
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

- (1) Pause, A. et al. (1994) *Nature* 371, 762-767.
- (2) Brunn, G.J. et al. (1997) *Science* 277, 99-101.
- (3) Gingras, A.C. et al. (1998) *Genes Dev.* 12, 502-513.
- (4) Fadden, P. et al. (1997) *J. Biol. Chem.* 272, 10240-10247.
- (5) Gingras, A.C. et al. (1999) *Genes Dev.* 13, 1422-1437.

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Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and U.S.S.N. 11/476,277) from Epitomics, Inc.

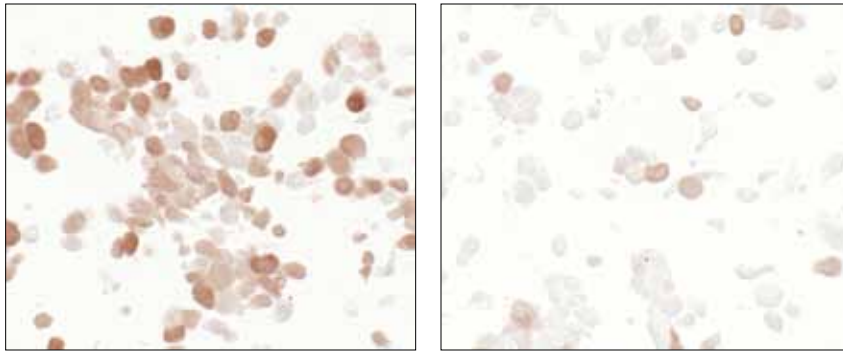


IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

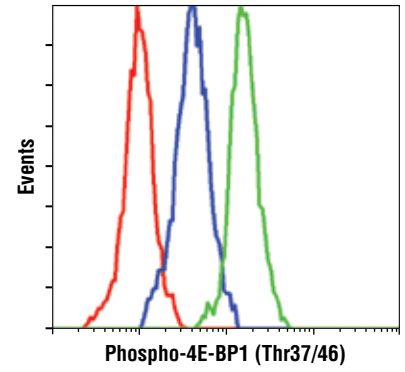
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

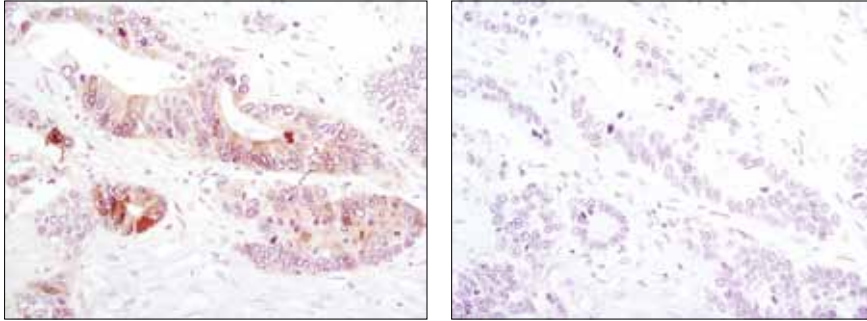
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



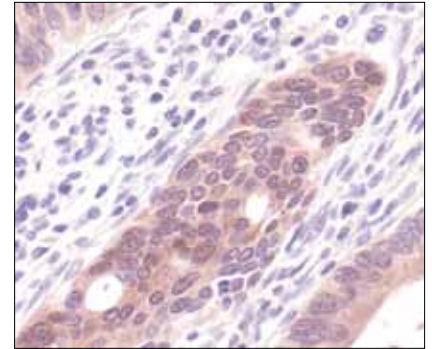
Immunohistochemical analysis of paraffin-embedded LNCaP cells untreated (left) or LY294002-treated (right) using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



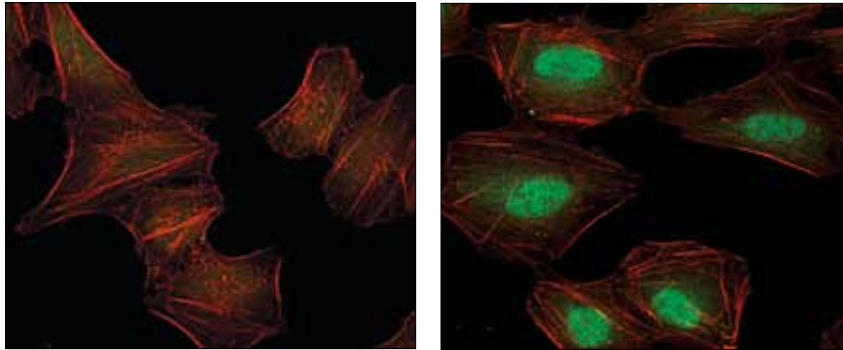
Flow cytometric analysis of Jurkat cells untreated (green), or LY294002, Wortmannin and U0126-treated (blue) using Phospho-4E-BP1 (Thr36/46) (236B4) Rabbit mAb compared to a nonspecific negative control antibody (red).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb #2855 in the presence of control peptide (left) or Phospho-4E-BP1 (Thr37/46) Blocking Peptide (right).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



Confocal immunofluorescent analysis of HeLa cells treated with LY294002 (left) or 20% serum (right) and labeled with Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor[®] 555 phalloidin (red).