Store at -20°C

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

#2855

www.cellsignal.com

Support: 877-678-TECH (8324)
www.cellsignal.com/support

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Entrez-Gene ID #1978
UniProt ID #Q13541

rev. 04/28/17

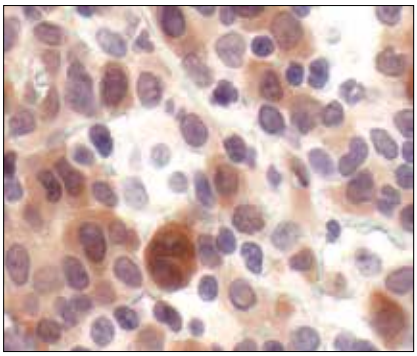
For Research Use Only. Not For Use In Diagnostic Procedures.

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

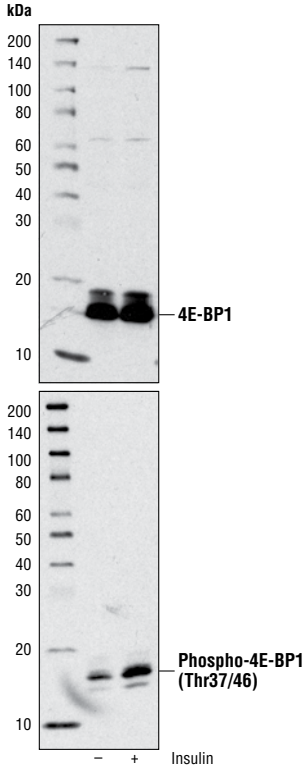
Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the translation initiation factor eIF4E. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt 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 at 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. Non-specific staining has been observed in mitotic cells by immunofluorescence.

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.

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 product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

(1) Pause, A. et al. (1994) *Nature* 371, 762-7.

(2) Brunn, G.J. et al. (1997) *Science* 277, 99-101.

(3) Gingras, A.C. et al. (1998) *Genes Dev* 12, 502-13.

(4) Fadden, P. et al. (1997) *J Biol Chem* 272, 10240-7.

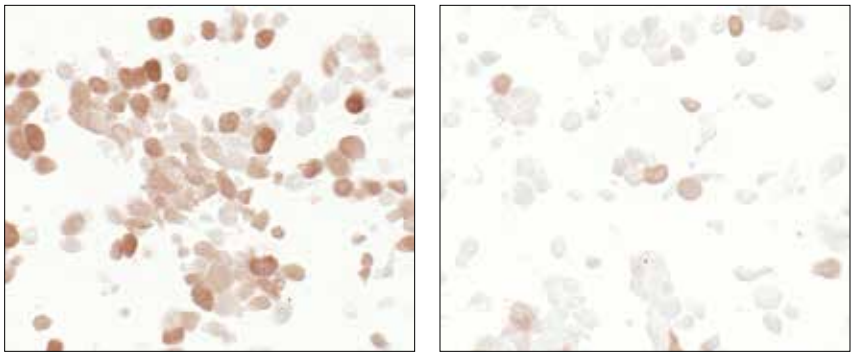
(5) Gingras, A.C. et al. (1999) *Genes Dev* 13, 1422-37.

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.

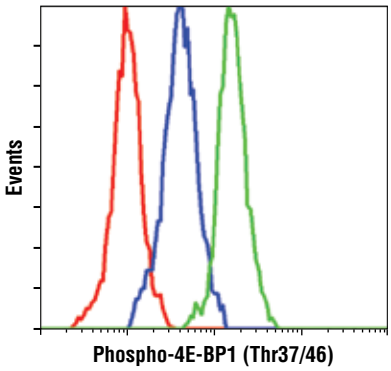
Thank you for your recent purchase. If you would like to provide a review visit www.cellsignal.com/comments.

Cell Signaling TECHNOLOGY®

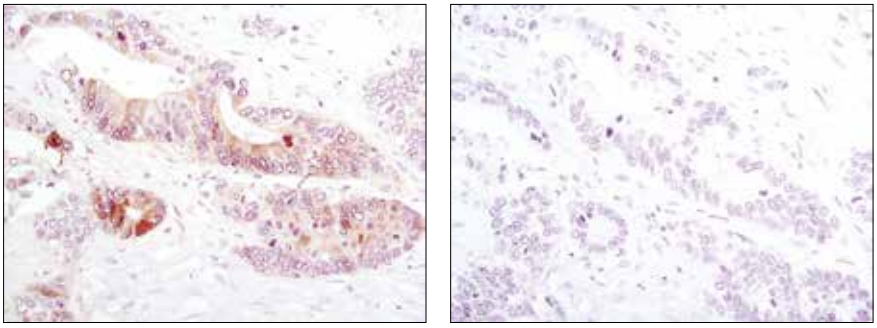
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



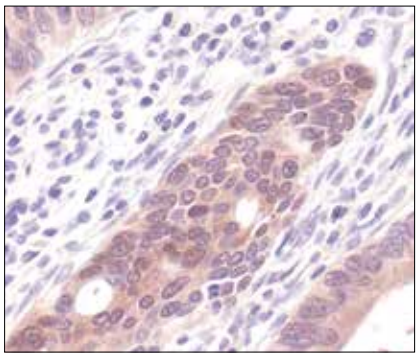
Immunohistochemical analysis of paraffin-embedded LNCaP cells untreated (left) or LY294002-treated (right) using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



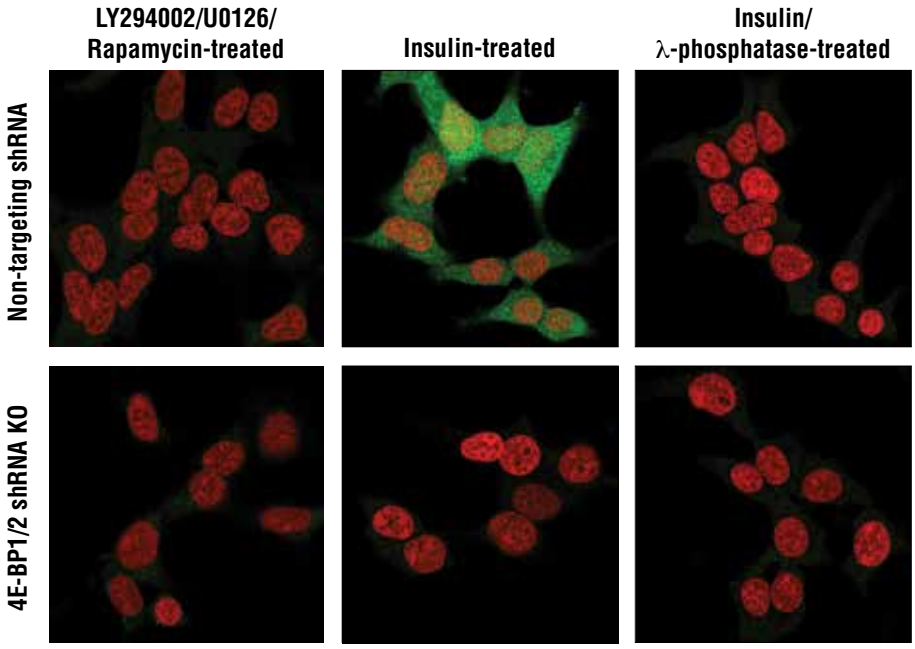
Flow cytometric analysis of Jurkat cells untreated (green), or LY294002, Wortmannin and U0126-treated (blue) using Phospho-4E-BP1 (Thr36/46) (236B4) Rabbit mAb compared to a nonspecific negative control antibody (red).



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 293 cells, expressing either non-targeting shRNA (top) or shRNA targeting 4E-BP1/2 (bottom), using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (green). To confirm phospho-specificity, cells were treated with an inhibitor cocktail consisting of LY294002 #9901, U0126 #9903, and Rapamycin #9904 (50 µM; 10 µm; 10 nM; 2 hr) (left), stimulated with insulin (100 nM, 30 min; middle), or processed with λ-phosphatase following insulin stimulation (right). Red = Propidium Iodide (PI)/RNase Staining Solution (#4087).