

# Phospho-4E-BP1 (Ser65) Antibody

- Small 100 µl (10 Western mini-blot)
- Large 300 µl (30 Western mini-blot)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

| Applications | Species Cross-Reactivity* | Molecular Wt. | Source   |
|--------------|---------------------------|---------------|----------|
| W, IP        | H, M, R, Mk               | 15-20 kDa     | Rabbit** |

**Background:** When bound to eIF4E, 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation. Upon hyperphosphorylation of 4E-BP1 this interaction is disrupted and cap-dependent translation is activated (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 (Ser65) Antibody detects endogenous levels of 4E-BP1 when phosphorylated at serine 65. This antibody has been shown to also recognize 4E-BP1 when phosphorylated at serine 101 (See application reference, Wang et al. 2003).

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide derived from the sequence of mouse 4E-BP1. Antibodies are purified by protein A and peptide affinity chromatography.

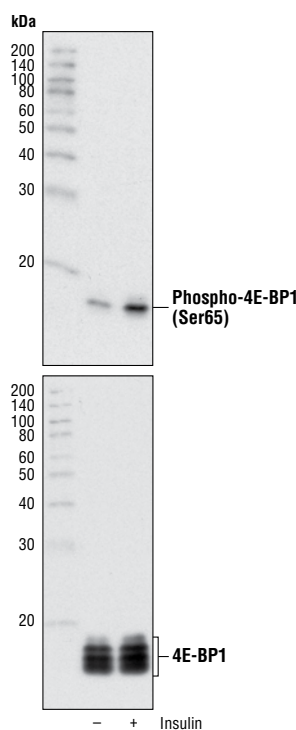
### Selected Application References:

Aoki, M. et al. (2001) A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. *Proc. Natl. Acad. Sci. USA* 98, 136–141. Application: W.

Jiang, Y.P. et al. (2001) Rapamycin-insensitive regulation of 4e-bp1 in regenerating rat liver. *J. Biol. Chem.* 276, 10943–10951. Application: W.

Wang, X. et al. (2003) The C Terminus of Initiation Factor 4E-Binding Protein 1 Contains Multiple Regulatory Features That Influence Its Function and Phosphorylation. *Mol. Cell Biol.* 23, 1546–1557. Application: W.

Gingras, A.C. et al. (2001) Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev.* 15, 2852–2864. Application: 2D Gel Electrophoresis.



Western blot analysis of extracts from 293 cells using 4E-BP1 Antibody #9644 (lower) and Phospho-4E-BP1 (Ser65) Antibody #9451 (upper). 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.

### 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.

**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.**

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 and 50% glycerol. 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:

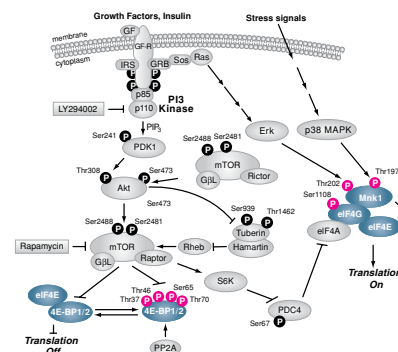
Western blotting 1:1000  
Immunoprecipitation 1:50

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

### Companion Products:

- 4E-BP1 Antibody #9452
- Nonphospho-4E-BP1 (Thr46) (87D12) Rabbit mAb #4923
- 4E-BP2 Antibody #2845
- Phospho-eIF4E (Ser209) Antibody #9741
- eIF4E Antibody #9742
- PathScan® Phospho-4E-BP1 (Thr37/Thr46) Sandwich ELISA Kit #7216
- 4E-BP Antibody Sampler Kit #9955
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.



**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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.