

Phospho-4E-BP1 (Ser65) Antibody

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

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

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

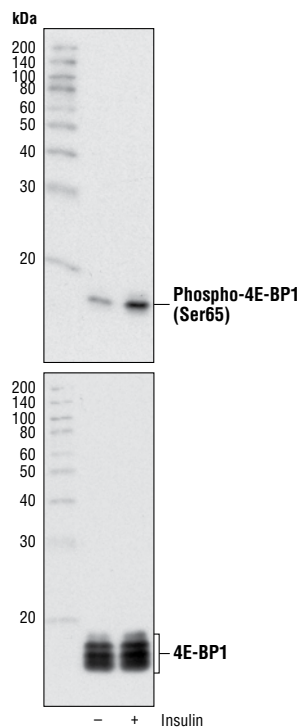
Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt 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 (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 phosphopeptide corresponding to the sequence of mouse 4E-BP1. Antibodies are purified by protein A and peptide affinity chromatography.

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.



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.

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.

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

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.