

Phospho-4E-BP1 (Ser65) (D9G1Q) Rabbit mAb



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Applications W, IP Endogenous	Species Cross-Reactivity* H, Mk	Molecular Wt. 15-20 kDa	Isotype Rabbit IgG**
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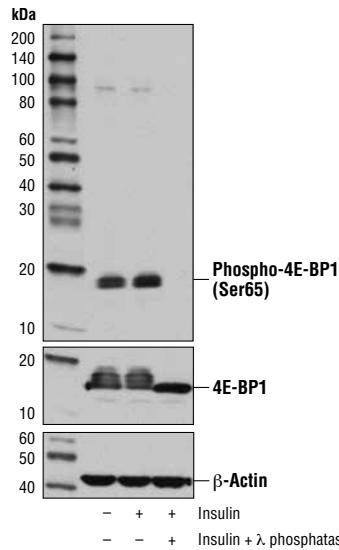
Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the translation initiation factor eIF4E. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt 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 (Ser65) (D9G1Q) Rabbit mAb recognizes endogenous levels of 4E-BP1 protein only when phosphorylated at Ser65.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser65 of human 4E-BP1 protein.

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.



Western blot analysis of extracts from serum starved HeLa cells, untreated (-), insulin-treated (1 μM, 30 min; +), or insulin and λ phosphatase-treated (+), using Phospho-4E-BP1 (Ser65) (D9G1Q) Rabbit mAb (upper), 4E-BP1 (53H11) Rabbit mAb #9644 (middle), and β-Actin (D6A8) Rabbit mAb #8457 (lower).

Entrez Gene ID #1978
UniProt ID #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
Immunoprecipitation	1:50

For product 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.

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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.