

Phospho-4E-BP1 (Ser65) (174A9) Rabbit mAb

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Entrez-Gene ID # 1978
UniProt ID #Q13541

Applications W, IP Endogenous	Species Cross-Reactivity* H, Mk, (C)	Molecular Wt. 15-20 kDa	Isotype Rabbit IgG**
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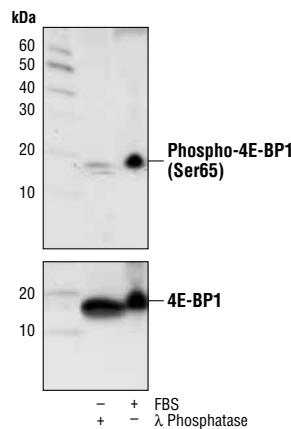
Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt 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) (174A9) Rabbit mAb detects endogenous levels of 4E-BP1 when phosphorylated at Ser65.

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

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, λ phosphatase treated, or treated with 20% FBS, using Phospho-4E-BP1 (Ser65) (174A9) Rabbit mAb (upper) or 4E-BP1 Antibody #9452 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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	1:100

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.

U.S. Patent No. 5,675,063

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.