

Phospho-eEF2 (Thr56) Antibody

✓ 100 µl
(10 Western mini-blots)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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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 Endogenous	H, M, R, Mk, C, Hm	95 kDa	Rabbit**

Background: Eukaryotic elongation factor 2 (eEF2) catalyzes the translocation of peptidyl-tRNA from the A site to the P site on the ribosome. It has been shown that phosphorylation of eEF2 at threonine 56 by eEF2 kinase inhibits its activity (1–4). eEF2 kinase is normally dependent on Ca²⁺ ions and calmodulin (5,6). eEF2 kinase can also be activated by PKA in response to elevated cAMP levels (7–9), which are generally increased in stress- or starvation-related conditions. A variety of treatments known to raise intracellular Ca²⁺ or cAMP levels have been shown to result in increased phosphorylation of eEF2, and thus to inhibit peptide-chain elongation. The inactive phosphorylated eEF2 can be converted to its active nonphosphorylated form by a protein phosphatase, most likely a form of protein phosphatase-2A (PP-2A). Insulin, which activates protein synthesis in a wide range of cell types, induces rapid dephosphorylation of eEF2 through mTOR signaling and may involve modulation of the activity of the PP-2A or the eEF2 kinase or both (10).

Specificity/Sensitivity: Phospho-eEF2 (Thr56) Antibody detects endogenous levels of eEF2 only when phosphorylated at Thr56. It does not recognize eEF2 phosphorylated at other sites.

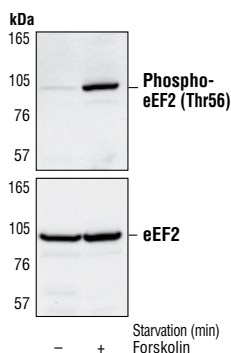
Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr56 of human eEF2. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:

Arora, S. et al. (2002) Detection of anti-elongation factor 2 kinase (calmodulin-dependent protein kinase III) antibodies in patients with systemic lupus erythematosus. *Biochem. Biophys. Res. Commun.* 293, 1073–1076. Application: W.

Background References:

- (1) Nairn, A.C. and Palfrey, H.C. (1987) *J. Biol. Chem.* 262, 17299–17303.
- (2) Ryazanov, A.G. et al. (1988) *Nature* 334, 170–173.
- (3) Carlberg, U. et al. (1990) *Eur. J. Biochem.* 191, 639–645.
- (4) Redpath, N.T. et al. (1993) *Eur. J. Biochem.* 213, 689–699.



Western blot analysis of extracts from C6 cells, untreated or forskolin-treated (10 µM for 60 minutes), using Phospho-eEF2 (Thr56) Antibody (upper) or eEF2 Antibody #2332 (lower).

- (5) Nairn, A.C. et al. (1985) *Proc. Natl. Acad. Sci. USA* 82, 7939–7943.
- (6) Palfrey, H.C. et al. (1987) *J. Biol. Chem.* 262, 9785–9792.
- (7) Redpath, N.T. and Proud, C.G. (1993) *Biochem. J.* 293, 31–34.
- (8) Diggie, T. et al. (1998) *Biochem. J.* 336, 525–529.
- (9) Hovland, R. et al. (1999) *FEBS Lett.* 444, 97–101.
- (10) Proud, C. (2000) *Translational Control of Gene Expression*. Cold Spring Harbor Laboratory Press, NY, 719–739.

Entrez-Gene ID # 1938
Swiss-Prot Acc. # P13639

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.

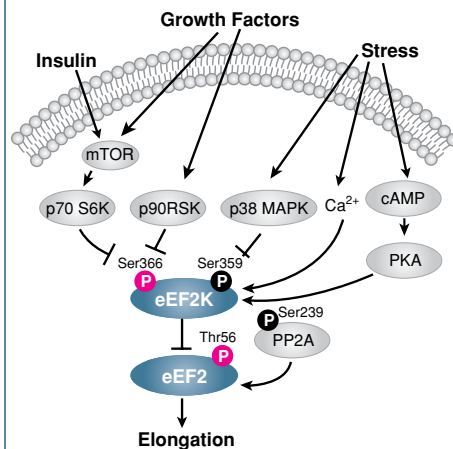
Recommended Antibody Dilutions:
Western Blotting 1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Companion Products:

- eEF2 Antibody #2332
- Phospho-eEF2k (Ser366) Antibody #3691
- eEF2k Antibody #3692
- Phospho-(Ser/Thr) PKA Substrate Antibody #9621
- Rapamycin (FRAP/mTOR Inhibitor) #9904
- 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
- 20X LumiGLO® Reagent and 20X Peroxide #7003

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



IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.