#9741 Store at –20°C

Phospho-eIF4E (Ser209) Antibody

☐ Small 100 µl
(10 Western mini-blots)

☐ Large 300 µl
(30 Western mini-blots)

Cell Signaling TECHNOLOGY®

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Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, M, R, Mk	25 kDa	Rabbit

Background: Eukaryotic initiation factor 4E (eIF4E) binds to the mRNA cap structure, thereby mediating the initiation of translation (1,2). eIF4E interacts with eIF4G, which serves as a scaffold protein for the assembly of eIF4E and eIF4A to form the eIF4F complex (2). eIF4B is thought to assist the eIF4F complex in translation initiation. Upon activation by mitogenic and/or stress stimuli mediated by Erk and p38 MAPK, Mnk1 has been shown to phosphorylate eIF4E at Ser209 in vivo (3,4). Two Erk and p38 MAPK phosphorylation sites have been identified in mouse Mnk1, Thr197 and Thr202, which are essential for Mnk1 kinase activity (3). The carboxy terminal region of eIF4G also contains serum-stimulated phosphorylation sites, including Ser1108, Ser1148 and Ser1192 (5). It is known that their phosphorylation is blocked by the PI3 kinase inhibitor LY294002 and by the FRAP/mTOR inhibitor rapamycin.

Specificity/Sensitivity: Phospho-eIF4E (Ser209) Antibody detects endogenous levels of eIF4E only when phosphorylated at serine 209.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH–coupled) corresponding to residues surrounding Ser209 of human eIF4E. Antibodies are purified by protein A and peptide affinity chromatography.

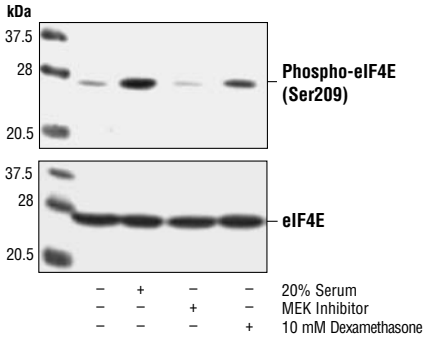
Selected Application References:

He, Y. et al. (2001) Regulation of mRNA translation and cellular signaling by hepatitis C virus nonstructural protein NS5A. *J. Virol.* 75, 5090–5098. Application: W.

Banerjee, S. et al. (2002) Murine coronavirus replication-induced p38 mitogen-activated protein kinase activation promotes interleukin-6 production and virus replication in cultured cells. *J. Virol.* 76, 5937–4948. Application: W.

Lindemann, S. et al. (2001) Integrins regulate the intracellular distribution of eukaryotic initiation factor 4E in platelets: A checkpoint for translational control. *J. Biol. Chem.* 276, 33947–33951. Application: W.

Pyronnet, S. et al. (2001) Suppression of cap-dependent translation in mitosis. *Genes Dev.* 15, 2083–2093. Application: W.



Western blot analysis of extracts from NIH/3T3 cells, untreated or treated with serum, PD98059 or Dexamethasone, using Phospho-eIF4E (Ser209) Antibody (upper) or eIF4E Antibody #9742 (lower).

Background References:

(1) Sonenberg, N. et al. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4843–4847.

(2) Gingras, A.C. et al. (1999) *Annu. Rev. Biochem.* 68, 913–963.

(3) Waskiewicz, A. et al. (1999) *Mol. Cell. Biol.* 19, 1871–1880.

(4) Pyronnet, S. et al. (1999) *EMBO J.* 18, 270–279.

(5) Raught, B. et al. (2000) *EMBO J.* 19, 434–444.

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.*

Recommended Antibody Dilutions:

Western blotting	1:1000

Companion Products:

Phospho-4E-BP1 (Ser65) Antibody #9451

Phospho-4E-BP1 (Thr70) Antibody #9455

eIF4E Antibody #9742

4E-BP1 (53H11) Rabbit mAb #9644

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb #2855

Nonphospho-4E-BP1 (Thr46) (87D12) Rabbit mAb #4923

Phospho-eIF4G (Ser1108) Antibody #2441

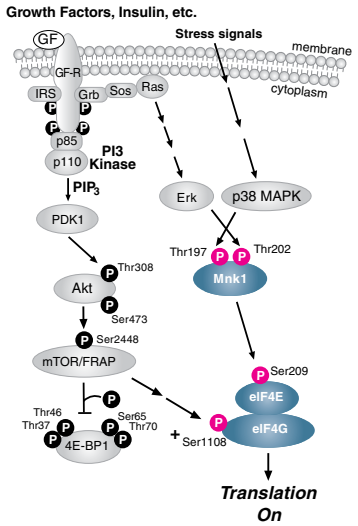
eIF4G Antibody #2498

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



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 **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.