Store at –20°C

#9742

eIF4E 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, IHC-P, IHC-F	H, M, R, Mk, Z	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: eIF4E Antibody detects endogenous levels of total eIF4E protein. The antibody does not cross-react with other proteins.

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

Selected Application References:

Krichevsky, A.M. and Kosik, K.S. (2001) Neuronal RNA granules: A link between RNA localization and stimulation-dependent translation. *Neuron* 32, 683–696. 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, 5937–5948. Application: W.

Background References:

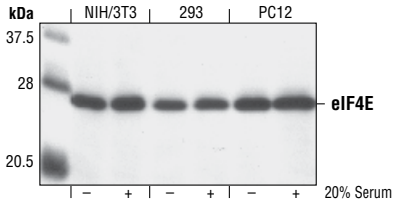
(1) Sonenberg, N. et al. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4843-4847.

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

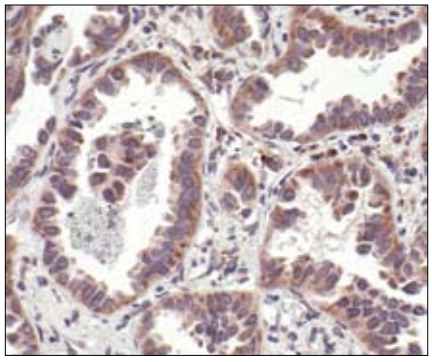
(3) Waskiewicz, A. et al. (1999) *Mol. Cell. Biol.* 19, 1871-1880.

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

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



Western blot analysis of extracts from control and serum treated NIH/3T3, 293 or PC12 cells, using eIF4E Antibody.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, showing cytoplasmic localization, using eIF4E Antibody.

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
Immunohistochemistry (Paraffin)	1:100
IHC Protocol:	Citrate/TBST
Immunohistochemistry (Frozen)	1:100
Fixative:	10% Neutral buffered formalin

Companion Products:

Phospho-eIF4E (Ser209) Antibody #9741

Phospho-eIF4G (Ser1108) Antibody #2441

eIF4G Antibody #2498

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

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

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

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

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

Phospho-Mnk1 (Thr197/202) Antibody #2111

PABP1 Antibody #4992

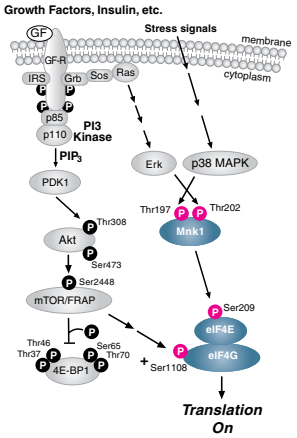
Anti-rabbit IgG, HRP-linked Antibody #7074

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

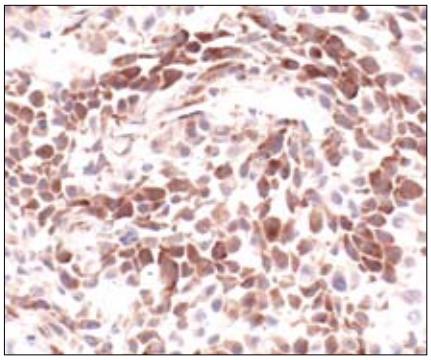


IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

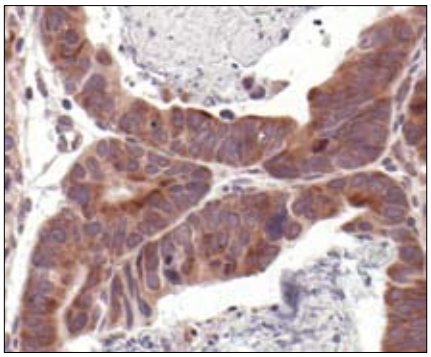
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

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

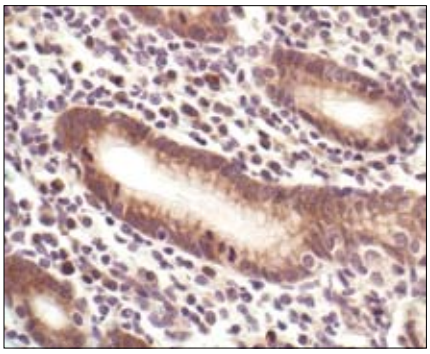
Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Immunohistochemical analysis of frozen H1650 xenograft, using eIF4E Antibody.



Immunohistochemical analysis of paraffin-embedded human renal cell carcinoma, using eIF4E Antibody.



Immunohistochemical analysis of paraffin-embedded human colon, using eIF4E Antibody in the presence of control peptide (left) or antigen-specific peptide (right).

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.

Immunohistochemistry Protocol (Paraffin)

***IMPORTANT:** See product data sheet for the appropriate wash buffer and antigen unmasking procedure.

- For Citrate/PBST, use steps 5a, 6a and C1.
- For Citrate/TBST, use steps 5b, 6a and C1.
- For EDTA/PBST, use steps 5a, 6b and C2.
- For EDTA/TBST, use steps 5b, 6b and C2.

A Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH_2O)
4. Hematoxylin (optional)
5. ***Wash Buffer:**

a. **For Citrate/PBST OR EDTA/PBST:** 1X PBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X PBS to 900 ml dH_2O. Add 1ml Tween-20 and mix. **10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phophate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 7.4.

b. **For Citrate/TBST OR EDTA/TBST:** 1X TBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X TBS to 900 ml dH_2O. Add 1 ml Tween-20 and mix. **10X Tris Buffered Saline (TBS):** To prepare 1 L add 24.2 g Trizma® base ($C_4H_{11}NO_3$) and 80 g sodium chloride (NaCl) to 1 L dH_2O. Adjust pH to 7.6 with concentrated HCl.

6. ***Antigen Unmasking Solution:**

a. **For Citrate/PBST OR Citrate/TBST:** 10 mM Sodium Citrate Buffer: To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.

b. **For EDTA/PBST OR EDTA/TBST:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 8.0.

c. **Alternative Unmasking: 10 mM Tris:** To prepare 1 L add 1.21 g Trizma® Base ($C_4H_{11}NO_3$) to 1 L dH_2O. Adjust pH to 10.0.

7. **3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H_2O_2 to 90 ml dH_2O.
8. **Blocking Solution:** 5% horse serum or goat serum diluted in recommended wash buffer.
9. Biotinylated secondary antibody.
10. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
11. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Deparaffinization/Rehydration

NOTE: Do not allow slides to dry at any time during this procedure.

NOTE: Consult product data sheet for recommended wash buffer.

1. **Deparaffinize/hydrate sections:**
   - **a.** Incubate sections in three washes of xylene for 5 minutes each.
   - **b.** Incubate sections in two washes of 100% ethanol for 10 minutes each.
   - **c.** Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH_2O for 5 minutes each.

C *Antigen Unmasking

NOTE: Consult product data sheet for specific recommendation for the unmasking solution.

1. **For Citrate/PBST OR Citrate/TBST:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
2. **For EDTA/PBST OR EDTA/TBST:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.
3. **Alternate:** Bring slides to a boil in 10 mM Tris pH 10.0 followed by 10 minutes at a sub boiling temperature. Cool slides on bench top for 30 minutes.

D Staining

1. Wash sections in dH_2O three times for 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections in dH_2O twice for 5 minutes each.

NOTE: Consult product data sheet for recommended wash buffer.

4. Wash section in wash buffer for 5 minutes.
5. Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100-400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
7. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
8. Add 100-400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
9. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
10. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
11. Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
12. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
13. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
14. As soon as the sections develop, immerse slides in dH_2O.
15. If desired, counterstain sections in hematoxylin per manufacturer's instructions.
16. Wash sections in dH_2O two times for 5 minutes each.
17. Dehydrate sections:
    - **a.** Incubate sections in 95% ethanol two times for 10 seconds each.
    - **b.** Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    - **c.** Repeat in xylene, incubating sections two times for 10 seconds each.
18. Mount coverslips.

IHC Frozen Section Protocol

A Solutions and Reagents

1. Xylene
2. Ethanol (anhydrous denatured, histological grade 100% and 95%)
3. Hematoxylin (optional)
4. **Fixative: For optimal fixative, please refer to the product data sheet**
   - **4a.** 10% Neutral buffered formalin
   - **4b.** Acetone
   - **4c.** Methanol
   - **4d.** 16% formaldehyde
     - **4d1. 3% formaldehyde:** To prepare, add 18.75 ml 16% formaldehyde to 81.25 ml 1X TBS.
5. **10X Tris Buffered Saline (TBS):** To Prepare 1 L add 24.2 g Trizma base ($C_4H_{11}NO_3$) and 80 g sodium chloride (NaCl) to 1 L dH_2O. Adjust pH to 7.6 with concentrated HCl.
6. **Wash buffer:** 1X Tris Buffered Saline (TBS) To prepare 1 L add 100 ml 10X TBS to 900 ml dH_2O.
7. **Methanol/Peroxidase:** To prepare, add 10 mL 30% H_2O_2 to 90 ml methanol. Store at -20°C**.**
8. **Blocking Solution:** 1X TBS/0.3% Triton-X 100/5% normal goat serum
   To prepare: add 500 µl goat serum and 30 µl Triton-X 100 to 9.5 ml 1X TBS.
9. **Biotinylated Secondary Antibody.**
10. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA). Prepare according to manufacturer's instructions 30 minutes before use**.**
11. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Sectioning

1. For tissue stored at -80°C: remove from freezer and equilibrate at -20°C for approximately 15 minutes before attempting to section. This may prevent cracking of the block when sectioning.
2. Section tissue at a range of 6-8 µm and place on positively charged slides.
3. Allow sections to air dry on bench for a few minutes before fixing (this helps sections adhere to slides).

C Fixation

NOTE: Consult product data sheet to determine the optimal fixative.

1. After sections have dried on the slide, fix in optimal fixative as directed below.
   - **1a. 10% Neutral buffered formalin:** 10 minutes at room temperature. Proceed with staining procedure immediately.
   - **1b. Cold acetone:** 10 minutes at –20°C. Air dry. Proceed with staining procedure immediately.
   - **1c. Methanol:** 10 minutes at –20°C. Proceed with staining procedure immediately.
   - **1d. 3% Formaldehyde:** 15 minutes at room temperature. Proceed with staining procedure immediately.
   - **1e. 3% Formaldehyde/methanol:** 15 minutes at room temperature, followed by 5 minutes in methanol at –20°C **(do not rinse in between)**. Proceed with staining procedure immediately.

D Staining

1. Wash sections in wash buffer twice for 5 minutes.
2. Incubate for 10 minutes in 3% H_2O_2 diluted in methanol at room temperature.
3. Wash sections in wash buffer twice for 5 minutes.
4. Block each section with blocking solution for one hour at room temperature.
5. Remove blocking solution and add 100-400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution). Incubate overnight at 4°C.
   **Refer to product datasheet to determine the recommended dilution.*
6. Remove antibody solution and wash sections three times with wash buffer for 5 minutes each.
7. Add 100-400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
8. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
9. Remove secondary antibody solution and wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 µl ABC reagent to each section and incubate for 30 min. at room temperature.
11. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
12. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
13. As soon as the sections develop, immerse slides in dH_2O.
14. If desired, counterstain sections in Hematoxylin per manufacturer's instructions.
15. Wash sections in dH_2O two times for 5 minutes each.
16. **Dehydrate sections:**
    - **16a.** Incubate sections in 95% ethanol two times for 10 seconds each.
    - **16b.** Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    - **16c.** Repeat in xylene, incubating sections two times for 10 seconds each.
17. Mount coverslips.