

# Phospho-eIF2 $\alpha$ (Ser51) Antibody

- Small 100  $\mu$ l  
(10 Western mini-blot)
- Large 300  $\mu$ l  
(30 Western mini-blot)

rev. 10/07/08

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.

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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, Mk, Dm, Sc	38 kDa	Rabbit**

**Background:** Phosphorylation of the  $\alpha$  subunit of eukaryotic initiation factor 2 is a well documented mechanism of downregulating protein synthesis under a variety of stress conditions. Eukaryotic initiation factor 2 binds GTP and Met-tRNAi and transfers Met-tRNA to the 40S subunit to form the 43S preinitiation complex (1,2). For eIF2 to promote a new round of translation initiation, GDP must be exchanged for GTP, a reaction catalyzed by eIF2B (1,2). Kinases activated by viral infection (PKR), endoplasmic reticulum stress (PERK/PEK), amino acid deprivation (GCN2) and hemin deficiency (HRI) can phosphorylate the alpha subunit of eIF2 (3,4). This phosphorylation stabilizes the eIF2-GDP-eIF2B complex, inhibiting the turnover of eIF2B. Induction of PKR by IFN- $\gamma$  and TNF- $\alpha$ , or stress provoked by depletion of endoplasmic reticulum calcium levels, induces potent phosphorylation of eIF2 $\alpha$  at Ser51 (5,6).

**Specificity/Sensitivity:** Phospho-eIF2 $\alpha$  (Ser51) Antibody detects endogenous eIF2 $\alpha$  only when phosphorylated at Ser51. The antibody does not recognize eIF2 $\alpha$  phosphorylated at other sites.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser51 of human eIF2 $\alpha$ . Antibodies are purified by protein A and peptide affinity chromatography.

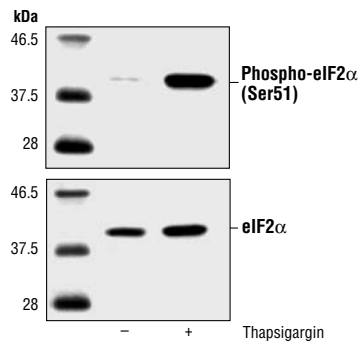
### Selected Application References:

Williams, D.D. et al. (2000) Characterization of the initiation factor eIF2B and its regulation in *Drosophila melanogaster*. *J. Biol. Chem.* 276, 3733–3742. Application: W.

Koumenis, C. et al. (2002) Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2 $\alpha$ . *Mol Cell Biol* 22, 7405–7416. Application: W.

Li, W. et al. (2004) Mutations linked to leukoencephalopathy with vanishing white matter impair the function of the eukaryotic initiation factor 2B complex in diverse ways. *Mol. Cell. Biol.* 24, 3295–3306. Application: W.

Connor, J.H. et al. (2001) Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1. *Mol. Cell. Biol.* 21, 6841–6850. Application: W.



Western blot analysis of extracts from PC12 cells, untreated or thapsigargin-treated (300 nM), using Phospho-eIF2 $\alpha$  (Ser51) Antibody (upper) or eIF2 $\alpha$  Antibody #9722 (lower).

### Background References:

- (1) Kimball, S.R. (1999) *Int. J. Biochem. Cell Biol.* 31, 25–29.
- (2) De Haro, C. et al. (1996) *FASEB J.* 10, 1378–1387.
- (3) Kaufman, R.J. (1999) *Genes Dev.* 13, 1211–1233.
- (4) Sheikh, M.S. and Fornace Jr., A.J. (1999) *Oncogene* 18, 6121–6128.
- (5) Cheshire, J.L. et al. (1999) *J. Biol. Chem.* 274, 4801–4806.
- (6) Zamanian-Daryoush, M. et al. (2000) *Mol. Cell. Biol.* 20, 1278–1290.

Entrez-Gene ID #1965

Swiss-Prot Acc. #P05198

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/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

### Companion Products:

Phospho-eIF2 $\alpha$  (Ser51) (119A11) Rabbit mAb #3597eIF2 $\alpha$  Antibody #9722

eIF2B-e Antibody #3595

GCN2 Antibody #3302

Phospho-PKR (Thr451) Antibody #3075

Phospho-PERK (Thr980) Antibody #3191

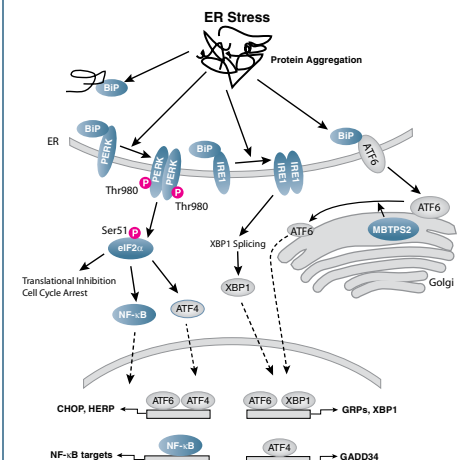
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

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



**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—ELISA 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—zebra fish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

## Western Immunoblotting Protocol (Primary Antibody 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.