

ER Stress Antibody Sampler Kit

✓ 1 Kit
(7 x 20 µl)



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For Research Use Only. Not For Use In Diagnostic Procedures.

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|--------------------------------------|-----------|----------|----------|-------------|
| BiP (C50B12) Rabbit mAb | 3177 | 20 µl | 78 kDa | Rabbit IgG |
| Calnexin (C5C9) Rabbit mAb | 2679 | 20 µl | 90 kDa | Rabbit IgG |
| Ero1-L α Antibody | 3264 | 20 µl | 60 kDa | Rabbit IgG |
| IRE1 α (14C10) Rabbit mAb | 3294 | 20 µl | 130 kDa | Rabbit IgG |
| CHOP (L63F7) Mouse mAb | 2895 | 20 µl | 27 kDa | Mouse IgG2a |
| PERK (D11A8) Rabbit mAb | 5683 | 20 µl | 140 kDa | Rabbit IgG |
| PDI (C81H6) Rabbit mAb | 3501 | 20 µl | 57 kDa | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |
| Anti-Mouse IgG, HRP-linked Antibody | 7076 | 100 µl | | Horse |

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The ER Stress Sampler Kit contains reagents to investigate ER stress within the cell. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Secretory and transmembrane proteins are synthesized on polysomes and translocate into the endoplasmic reticulum (ER) where they are often modified by the formation of disulfide bonds, amino-linked glycosylation and folding. The ER contains a pool of molecular chaperone proteins including calnexin, BiP and protein disulfide isomerase (PDI). Calnexin is an ER membrane, calcium-binding protein that retains newly synthesized glycoproteins inside the ER to ensure proper folding and quality control (1,2). Irregular protein folding within the ER increases BiP synthesis, which binds misfolded proteins to prevent them from forming aggregates and to assist them to refold properly (3).

PDI catalyzes the formation and isomerization of disulfide bonds required for a protein to reach its native state (4). Studies have found that the resident ER protein endoplasmic oxidoreductin-1 (Ero1) provides oxidizing potential to the ER in *Saccharomyces cerevisiae* (5). Ero1-L α is an ER membrane-associated N-glycoprotein that promotes oxidative protein folding (6). Disruptions of ER homeostasis leads to the accumulation of unfolded proteins. The ER has developed an adaptive mechanism called the unfolded protein response (UPR) to counteract compromised protein folding (7). This is regulated by

proteins such as the membrane-bound transcription factor protease site 2 (MBTPS2) and the serine/threonine kinase IRE1 (8-12). The PERK eIF2 α kinase is an ER resident transmembrane protein that couples ER stress signals to translation inhibition. ER stress increases PERK activity, which phosphorylates eIF2 α to reduce protein translation. PERK activation during ER stress correlates with autophosphorylation of its cytoplasmic kinase domain (13,14). Phosphorylation of PERK at Thr980 can serve as a marker for its activation status.

During ER stress, the level of CHOP expression is elevated and CHOP functions to mediate programmed cell death (15).

Specificity/Sensitivity: Each antibody in the ER Stress Antibody Sampler Kit detects endogenous levels of its target protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu156 of human PERK protein, the sequence around Gly584 of human BiP, the sequence around His963 of human IRE1 α , the sequence of human PDI and the sequence of human CHOP. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a sequence around Ala51 of human calnexin, the sequence around Leu218 of human Ero1-L α , and the sequence of mouse MBTPS2. Antibodies are purified by protein A and peptide affinity chromatography.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Background References:

- (1) Bergeron, J.J. et al. (1994) *Trends Biochem. Sci.* 19, 124-128.
- (2) Williams, D.B. (2006) *J. Cell Sci.* 119, 615-623.
- (3) Kohno, K. et al. (1993) *Mol. Cell. Biol.* 13, 877-890.
- (4) Ellgaard, L. and Ruddock, L.W. (2005) *EMBO Rep.* 6, 28-32.
- (5) Frand, A.R. and Kaiser, C.A. (1998) *Mol. Cell* 1, 161-170.
- (6) Cabibbo, A. et al. (2000) *J. Biol. Chem.* 275, 4827-4833.
- (7) Kaufman, R.J. et al. (2002) *Nat. Rev. Mol. Cell Biol.* 3, 411-421.
- (8) Nikawa, J. and Yamashita, S. (1992) *Mol. Microbiol.* 6, 1441-1446.
- (9) Cox, J.S. et al. (1993) *Cell* 73, 1197-1206.
- (10) Mori, K. et al. (1993) *Cell* 74, 743-756.
- (11) Lee, K. et al. (2002) *Genes Dev.* 16, 452-466.
- (12) Shen, J. and Prywes, R. (2004) *J. Biol. Chem.* 279, 43046-43051.
- (13) Harding, H.P. et al. (1999) *Nature* 397, 271-274.
- (14) Shi, Y. et al. (1998) *Mol. Cell. Biol.* 18, 7499-7509.
- (15) Zinszner, H. et al. (1998) *Genes Dev* 12, 982-95.

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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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.