

ER Stress Antibody Sampler Kit

✓ 1 Kit
(7 x 40 µl)

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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.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
BiP (C50B12) Rabbit mAb	3177	40 µl	78 kDa	Rabbit IgG
Calnexin (C5C9) Rabbit mAb	2679	40 µl	90 kDa	Rabbit IgG
Ero1-L α Antibody	3264	40 µl	60 kDa	Rabbit IgG
IRE1 α (14C10) Rabbit mAb	3294	40 µl	130 kDa	Rabbit IgG
CHOP (L63F7) Mouse mAb	2895	40 µl	27 kDa	Mouse IgG2a
PERK (C33E10) Rabbit mAb	3192	40 µl	140 kDa	Rabbit IgG
PDI (C81H6) Rabbit mAb	3501	40 µl	57 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-Mouse IgG, HRP-linked Antibody	7076	50 µl		Horse

See www.cellsignaling.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 contains enough primary and secondary antibodies to perform four Western blot experiments per 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).

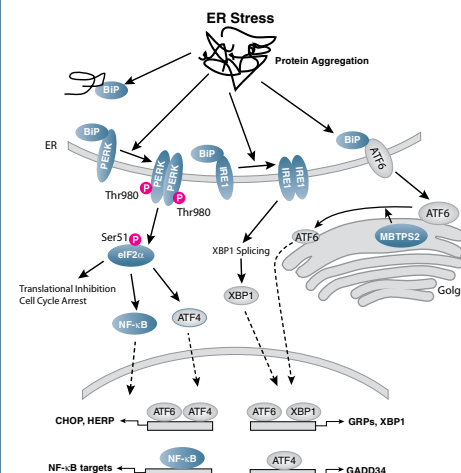
Specificity/Sensitivity: Each antibody in the ER Stress Antibody Sampler Kit detects endogenous levels of its target protein.

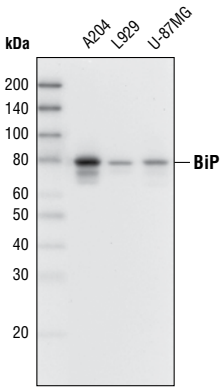
Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to the sequence of human PERK, the sequence around Gly584 of human BiP, the sequence around His963 of human IRE1 α , the sequence of human PDI and the sequence of human CHOP. Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from 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.

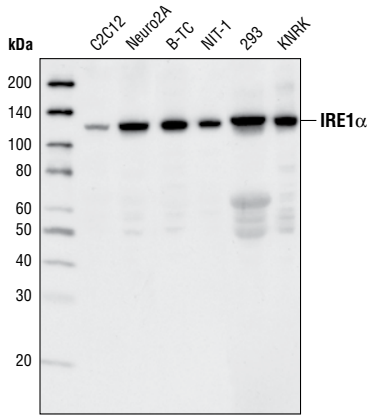
Recommended Antibody Dilutions:
Western blotting 1:1000

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

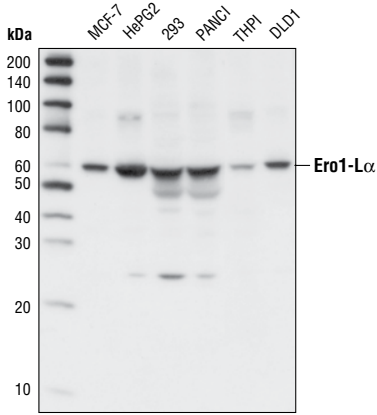




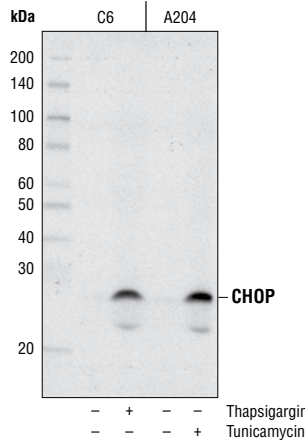
Western blot analysis of extracts from various cell lines using **BiP (C50B12) Rabbit mAb #3177**.



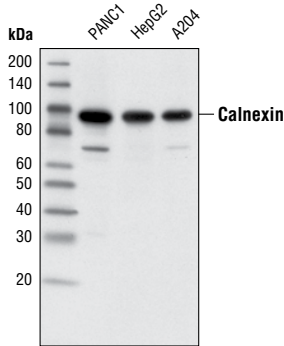
Western blot analysis of extracts from various cell lines, using **IRE1α (14C10) Rabbit mAb #3294**.



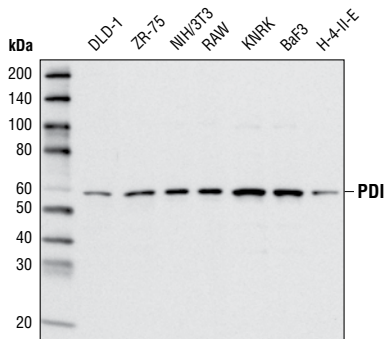
Western blot analysis of extracts from various cell lines, using **Ero1-Lα Antibody #3264**.



Western blot analysis of extracts from C6 and A204 cells, untreated or treated with thapsigargin (300 nM) or tunicamycin (24 μg/ml), using **CHOP (L63F7) Mouse mAb #2895**.



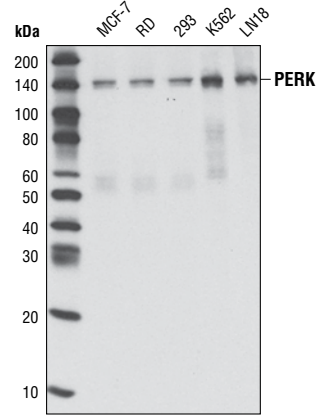
Western blot analysis of extracts from PANC1, HepG2 and A204 cells using **Calnexin (C5C9) Rabbit mAb #2679**.



Western blot analysis of extracts from various cell types using **PDI (C81H6) Rabbit mAb #3501**.

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.



Western blot analysis of extracts from various cell types using **PERK (C33E10) Rabbit mAb #3192**.

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[®]-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.
- 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 cm²) 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[®] (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.

- 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.