

**#4759** Store at -20°C

# ER Protein Folding Antibody Sampler Kit

1 Kit  
 (7 x 40 µl)

**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Source
BiP (C50B12) Rabbit mAb	3177	40 µl	78 kDa	Rabbit
Ero1-L $\alpha$ Antibody	3264	40 µl	60 kDa	Rabbit
ERp44 (D17A6) XP® Rabbit mAb	3798	40 µl	44 kDa	Rabbit
ERp57 (G117) Antibody	2881	40 µl	57 kDa	Rabbit
ERp72 Antibody	5033	40 µl	72 kDa	Rabbit
Grp94 Antibody	2104	40 µl	100 kDa	Rabbit
PDI Antibody	3501	40 µl	57 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

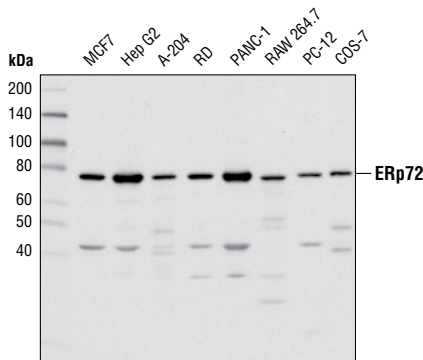
See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

**Description:** The ER Protein Folding Antibody Sampler Kit contains reagents to investigate the initiation of translation within the cell. The kit contains enough primary and secondary antibodies to perform four western blot experiments per primary antibody.

**Background:** After their synthesis, secretory proteins translocate into the endoplasmic reticulum (ER) where they are post-translationally modified and properly folded. To reach their native conformation, many secretory proteins require the formation of intra- or inter-molecular disulfide bonds (1). This process is called oxidative protein folding. Disulfide isomerase (PDI) catalyzes the formation and isomerization of these disulfide bonds (2). Studies on mechanisms of oxidative folding suggest that molecular oxygen oxidizes the ER-protein Ero1, which in turn oxidizes PDI through disulfide exchange (3). This event is then followed by PDI-catalyzed disulfide bond formation on folding proteins (3). Other ER resident proteins that possess the thioredoxin homology domains, including endoplasmic reticulum stress proteins 72, 57 and 44 (ERp72, ERp57 and ERp44), constitute the PDI family (4,5,6). The ER also contains a pool of molecular chaperones, including Grp94, to help proteins fold properly. Grp94 is a glucose-regulated protein (7) with sequence homology to Hsp90 (8). BiP is another chaperone whose synthesis is increased when protein folding is disturbed. BiP binds to misfolded proteins to prevent them from forming aggregates and assists in proper refolding (9).

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

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Gly584 of human



Western blot analysis of extracts from various cell lines using ERp72 (D70D12) XP® Rabbit mAb #5033.

BiP, the sequence of human ERp44, the residues surrounding Met279 of human ERp72 protein and the sequence of human PD1. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequences around Gly117 of human ERp57, Met622 of human Grp94 and Leu218 of human Ero1-L $\alpha$ . Polyclonal 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

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

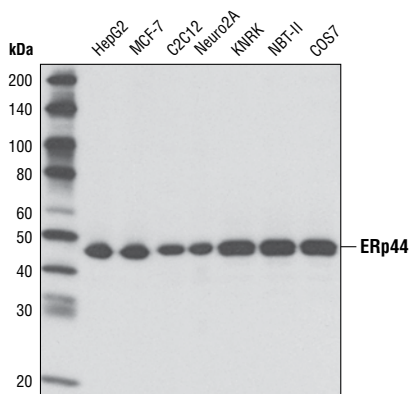
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

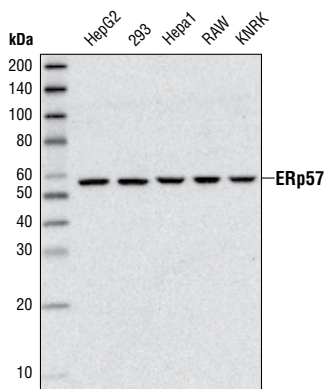
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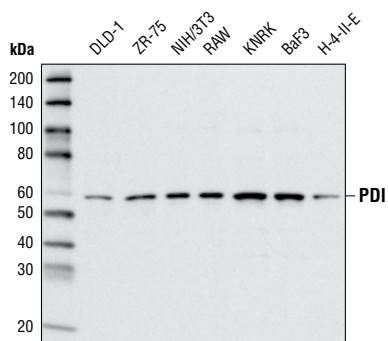
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus 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% sequence homology.



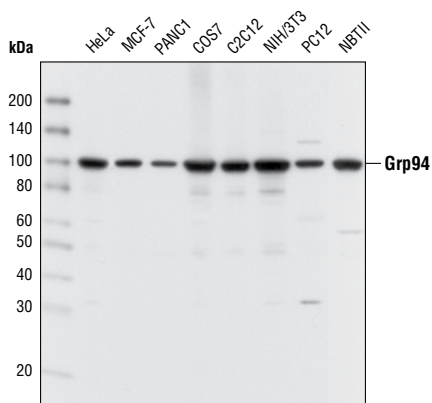
Western blot analysis of extracts from various cell lines using ERp44 (D17A6) XP® Rabbit mAb #3798.



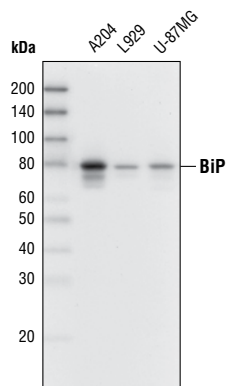
Western blot analysis of extracts from various cell types using ERp57 (G117) Antibody #2881.



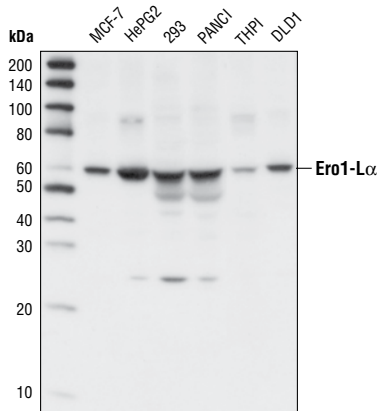
Western blot analysis of extracts from various cell lines using PDI (C81H6) Rabbit mAb #3501.



Western blot analysis of extracts from various cell types using Grp94 Antibody #2104.



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



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

## 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.