Kit Includes	Quantity	Applications	Reactivity	MW (kDa)	Isotype
BiP (C50B12) Rabbit mAb #3177	40 µl	W IHC-P IHC-F	H M	78	Rabbit IgG
Calnexin (C5C9) Rabbit mAb #2679	40 µl	W IHC-P IF-IC	H Mk	90	Rabbit
Ero1-Lα Antibody #3264	40 µl	W	H	60	Rabbit
IRE1α (14C10) Rabbit mAb #3294	40 µl	W IP	H M	130	Rabbit IgG
PDI (C81H6) Rabbit mAb #3501	40 µl	W IHC-P IF-IC	H M R Mk	57	Rabbit
CHOP (L63F7) Mouse mAb #2895	40 µl	W IP IF-IC	H M R	27	Mouse IgG2a
PERK (D11A8) Rabbit mAb #5683	40 µl	W IP IHC-P	H	140	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody #7074	100 µl				Goat
Anti-mouse IgG, HRP-linked Antibody #7076	100 µl				Horse

Applications Key: W=Western Blotting IP=Immunoprecipitation IHC-P=Immunohistochemistry (Paraffin) IHC-F=Immunohistochemistry (Frozen) IF-IC=Immunofluorescence (Immunocytochemistry)

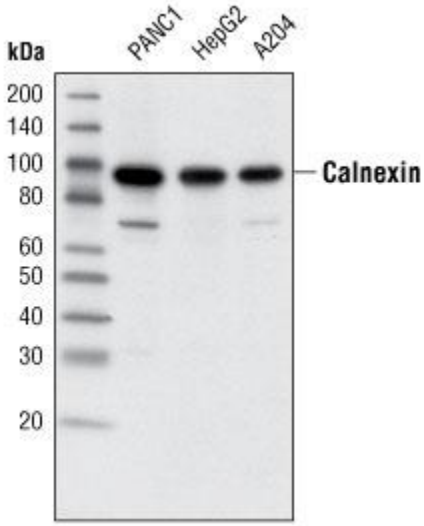
Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey

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

Specificity / Sensitivity

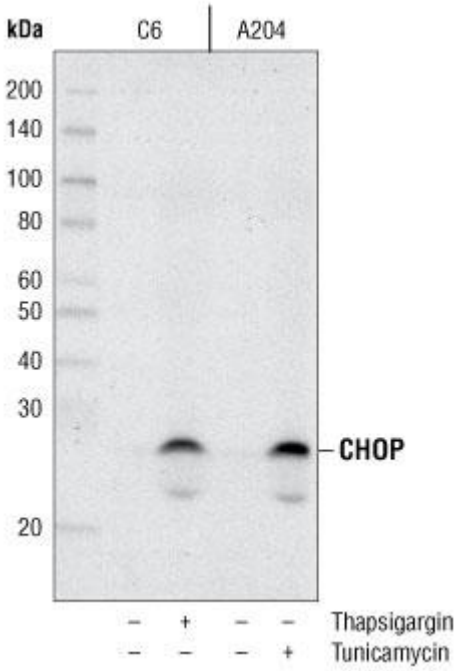
Each antibody in the ER Stress Antibody Sampler Kit detects endogenous levels of its target protein.

Western Blotting



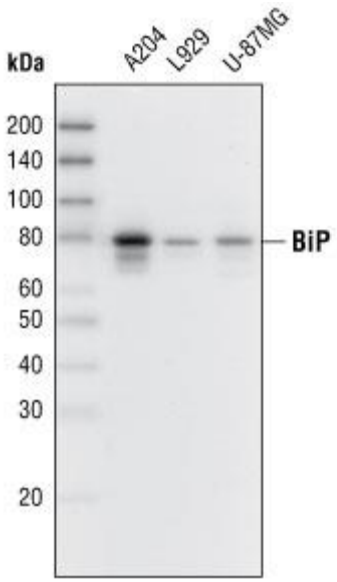
Western blot analysis of extracts from PANC1, HepG2 and A204 cells using Calnexin (C5C9) Rabbit mAb #2679.

Western Blotting



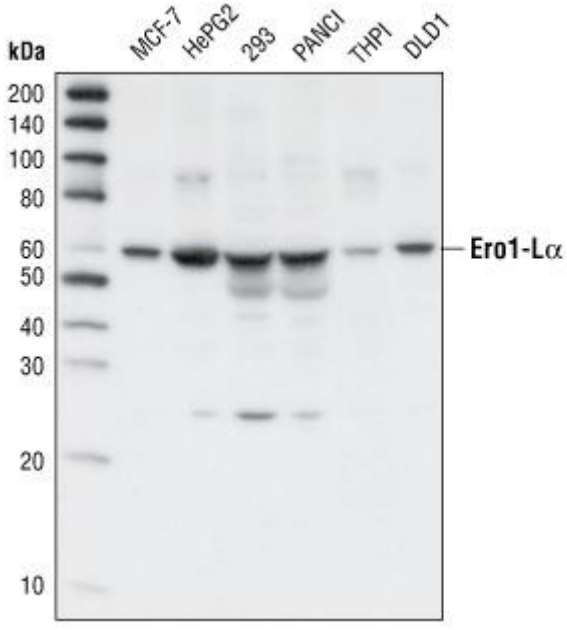
Western blot analysis of extracts from C6 and A204 cells, untreated or treated with thapsigargin (300 nM) or tunicamycin (24 µg/ml), using CHOP (L63F7) Mouse mAb #2895.

Western Blotting



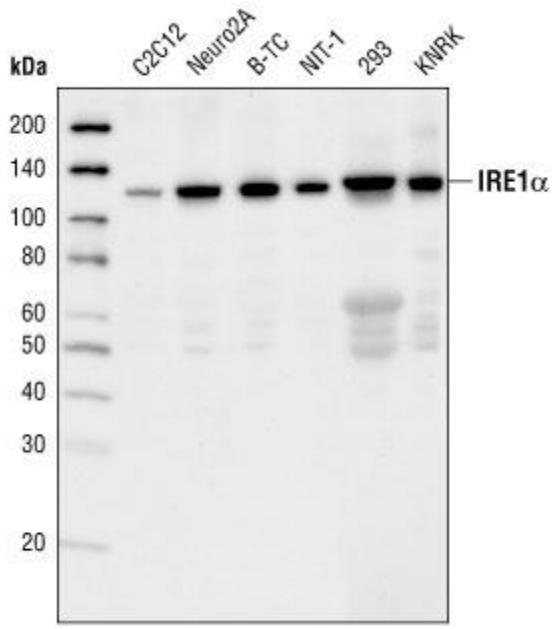
Western blot analysis of extracts from various cell lines using BiP (C50B12) Rabbit mAb #3177.

Western Blotting



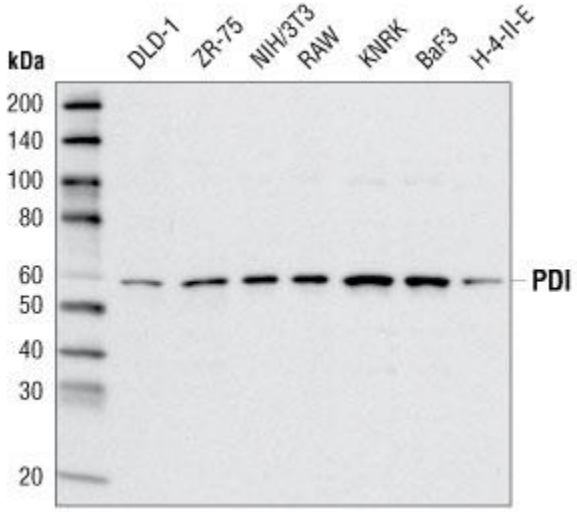
Western blot analysis of extracts from various cell lines using Ero1-Lα Antibody #3264.

Western Blotting



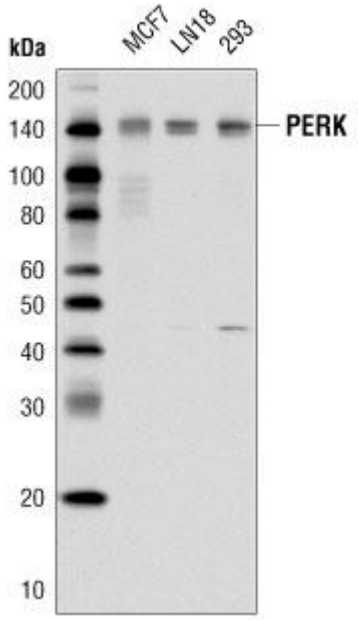
Western blot analysis of extracts from various cell lines using IRE1α (14C10) Rabbit mAb #3294.

Western Blotting



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

Western Blotting



Western blot analysis of extracts from various cell lines using PERK (D11A8) Rabbit mAb #5683.

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.

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 IRE1α, 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-Lα, and the sequence of mouse MBTPS2. Antibodies are purified by protein A and peptide affinity chromatography.

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-Lα 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 eIF2α kinase is an ER resident transmembrane protein that couples ER stress signals to translation inhibition. ER stress increases PERK activity, which phosphorylates eIF2α 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).

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.