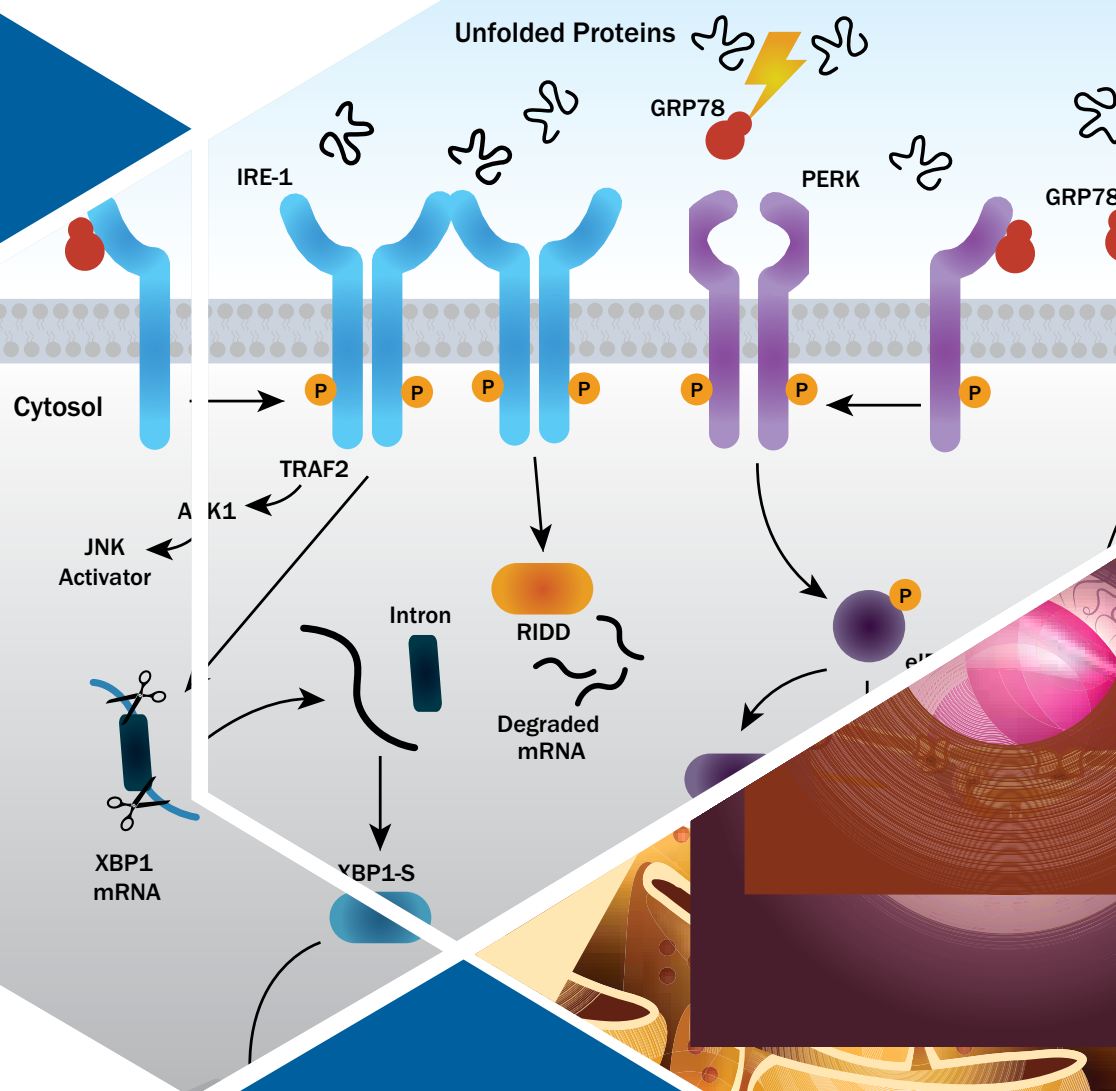


Antibodies for Unfolded Protein Response



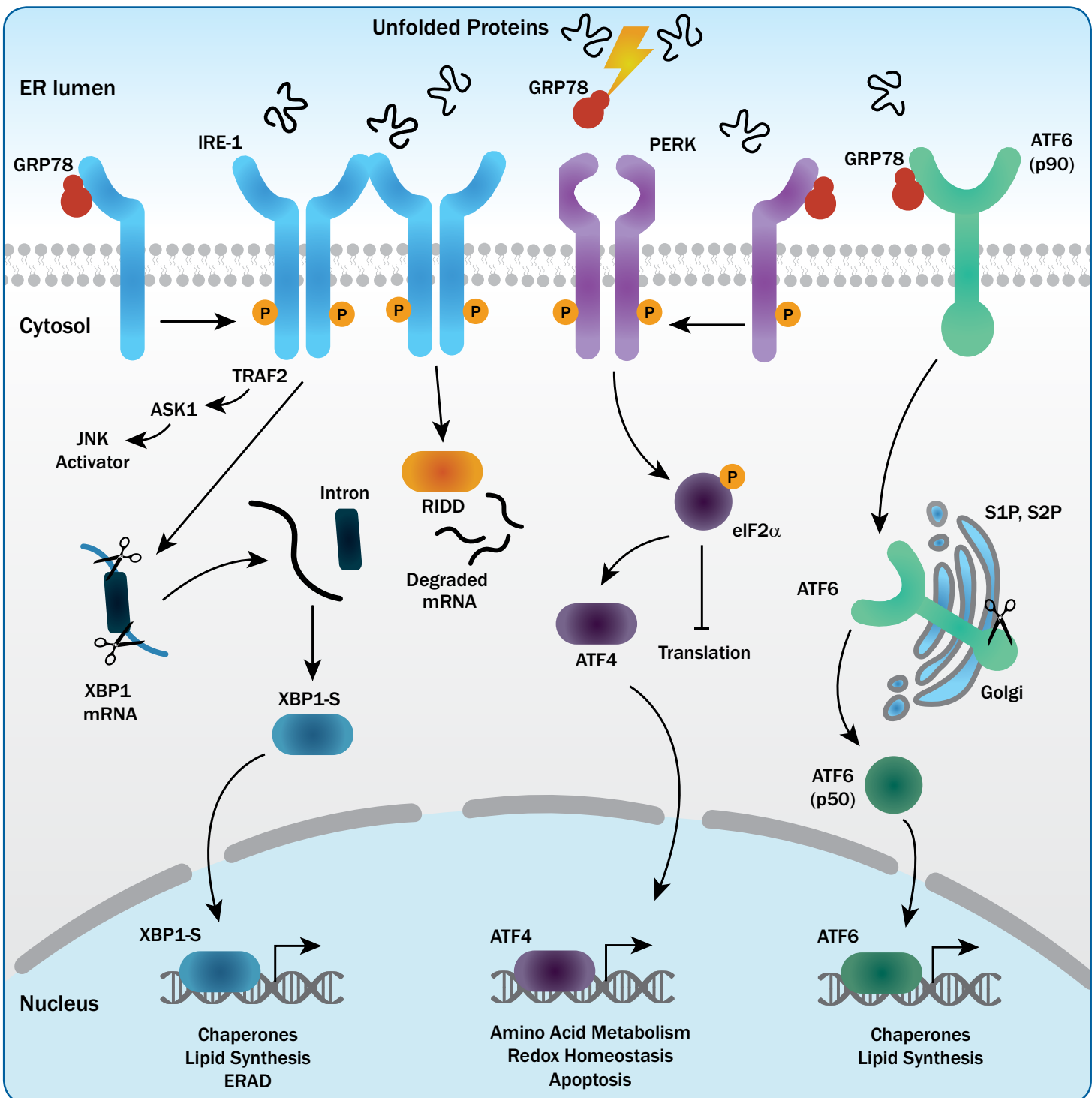
INTRODUCTION

The unfolded protein response (UPR) is a signaling mechanism activated in eukaryotic cells in response to endoplasmic reticulum (ER) stress, an accumulation of unfolded proteins in the ER lumen. Conditions such as high protein demand, viral infection, mutant protein expression, hypoxia, energy deprivation, or exposure to excessive oxidative stress can trigger UPR or ER stress. UPR serves three major functions: (i) blocking protein translation to restore homeostasis; (ii) positive regulation of protein folding related molecular chaperones; and (iii) up-regulation of signaling pathways responsible for targeting mis-/un-folded proteins in ER for ubiquitination mediated degradation. When ER stress is not relieved, UPR can induce apoptosis and cause cell death. A sustained over-activation of UPR is known to be involved in many human diseases including cancer, hyperglycemia, autoimmune conditions, hepatic disorders, retinopathies, acute lung injury and neuro-degeneration. Accordingly, a complete mechanistic understanding of UPR signaling is critical for the evaluation of its biological effects in normal and/or disease conditions, and for developing preventive as well as therapeutic measures against UPR associated diseases.

Regulation of UPR Signaling

UPR signaling is regulated via three major effector proteins which localize to the ER: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK). These proteins are maintained in their inactive/membrane bound state through binding to glucose response protein 78 (GRP78/BiP). Upon interaction with unfolded proteins, GRP78 is released from the UPR effector proteins, which leads to their activation. UPR is generally activated by an orchestrated interplay of all three arms of the ER stress signaling (see the pathway image).

UPR induction in mammalian cells

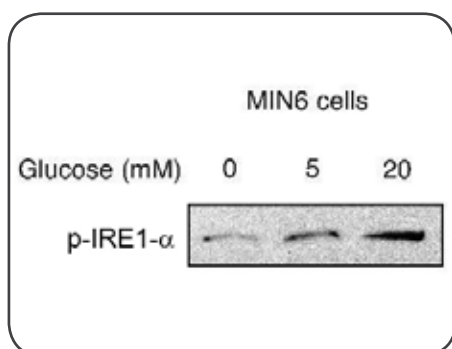


Inositol-Requiring Enzyme 1 Alpha (IRE1 Alpha)

IRE1 alpha, predicted mol. wt. 109.7 kDa, is an ER resident protein which is expressed ubiquitously in all tissue types. It is a single-pass type I membrane protein which localizes to the ER lumen, and interacts with several other proteins, including GRP78, DAB2IP, TRAF2, and TAOK3. Upon UPR activation, IRE1 alpha undergoes dimerization/auto-phosphorylation mediated activation (e.g. phospho-Ser724 IRE1 alpha). The active form of IRE1 alpha then induces the splicing of mRNA encoding the transcription factor, XBP1. Removal of an intron from XBP1 leads to the expression of an active form of XBP1 (XBP1-S, the spliced form) which positively regulates ER chaperones, as well as genes coding for the ER-associated protein degradation (ERAD) pathway and lipid metabolism.

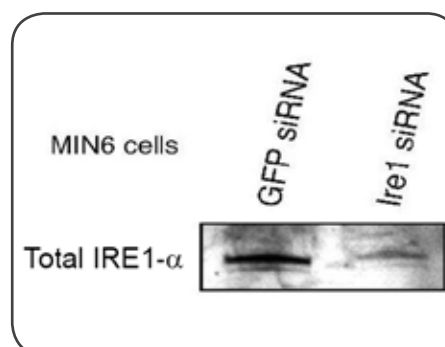
Through XBP1-independent pathways, IRE1 binds to tumour necrosis factor (TNF) receptor-associated factor 2 (TRAF2) and induces JUN amino-terminal kinase (JNK) activation. This interaction is known to modulate autophagic and apoptotic cell death. IRE1's endo-ribonuclease activity also induces Regulated IRE1-Dependent mRNA Decay (RIDD) which is implicated in lipid anabolism and apoptosis.

IRE1 alpha is also involved in the processes of cell cycle arrest, insulin metabolism, angiogenesis, and regulation of macro-autophagy.



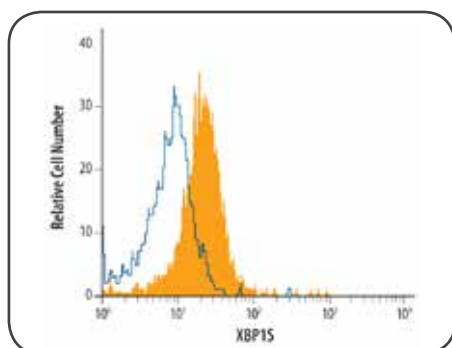
pSer724 IRE1 alpha Antibody [NB100-2323*]

Western blot (WB) detection of pSer724 IRE1 alpha Min6 cells treated with increasing concentrations of glucose for 3 hours prior to lysate preparation.



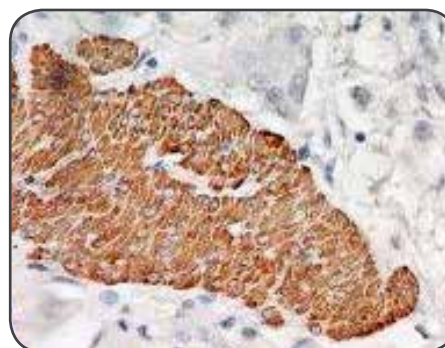
IRE1 alpha Antibody (Total) [NB100-2324]

WB detection of total IRE1 alpha protein in lysates from Min6 cells which were transfected with GFP-siRNA or Ire1-siRNA.



XBP1 Antibody (S Isoform) [MAB4257]

FLOW analysis of PFA fixed and saponin permeabilized RPMI 8226 human multiple myeloma cells using XBP1S antibody (filled histogram) or isotype control (MAB002, open histogram) with APC-conjugated secondary antibody (F0101B).



TRAF2 Antibody (33A1293) [NB100-56715]

IHC-P analysis of a formalin-fixed tissue section of human transitional cell carcinoma of the urinary bladder. Signal was developed using HRP-DAB method and the nuclei were counterstained using hematoxylin.

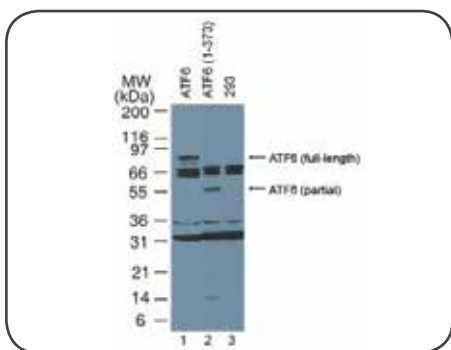
***Most cited phospho-IRE1 alpha antibody among offerings from all antibody companies.**

Activating Transcription Factor 6 (ATF6)

ATF6 is a transmembrane glycoprotein and transcription activator, which functions to initiate the UPR during ER stress. The predicted molecular weight of the canonical form of ATF6 is 74.5 kDa. However, ATF6 may be detected at approximately 90 kDa in Western blot, as the protein often undergoes glycosylation, forming the p90 version of ATF6.

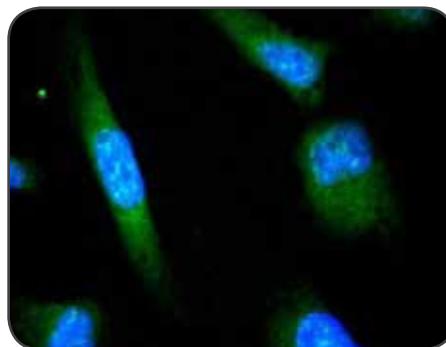
Upon sensing of ER stress, p90 is transported to the Golgi apparatus, where it is processed through site 1 protease (S1P) and S2P protease, releasing the N-terminal processed cAMP-dependent ATF-6 alpha form (cytosolic/cleaved-ATF6, p50). The cleaved p50 form of ATF6 then translocates to the nucleus of the cell, where it binds DNA on the ER stress response element (ERSE) for regulation of ER-associated protein degradation (ERAD) and UPR genes.

In addition to mediating the stress response through ERSE and ERAD, p50 also regulates transcription of the XBP1 protein, as well as the induction of apoptosis, regulation of transcription from RNA polymerase II promoter, ocular tissue development and visual perception.



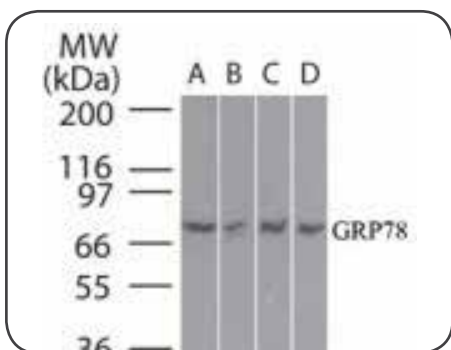
ATF6 Antibody (70B1413.1) [NBP1-40256]

WB analysis of HEK293 cells transfected with full-length ATF6, or with partial ATF6 protein (AA 1-373), and the non-transfected control cells.



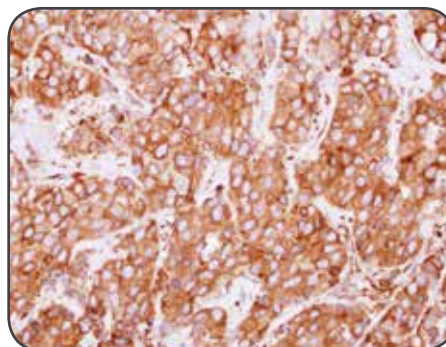
ATF6 Antibody [NBP1-75478]

ICC/IF analysis of HeLa cells using ATF6 antibody with detection employing FITC conjugated secondary (green). Nuclei were counterstained with DAPI (blue).



GRP78/HSPA5 Antibody [NB100-56411]

WB analysis of lysates from HeLa cells (A), human liver (B), mouse liver (C), and rat liver (D) tissues using GPR78 antibody with detection employing HRP labelled secondary and PicoTect ECL substrate.



ERp72 Antibody [NBP2-16371]

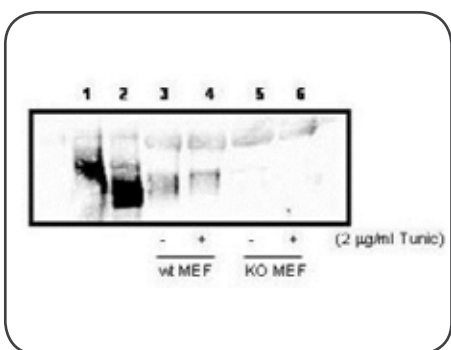
IHC analysis of a formalin fixed and paraffin-embedded tissue section of breast cancer using ERp72 antibody with HRP-DAB detection. Nuclei were counterstained using hematoxylin.

Protein kinase RNA-like ER Kinase (PERK)

PERK, predicted molecular weight 125.2 kDa, is a transmembrane protein kinase belonging to the PEK family of proteins, and is best known for its role in insulin processing.

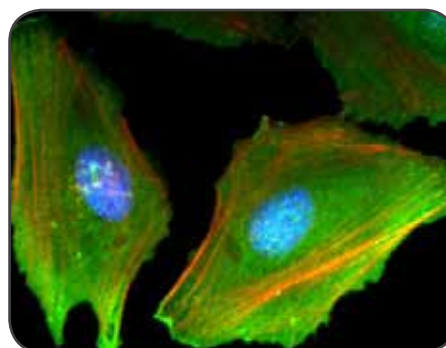
During ER stress responses and activation of the UPR, PERK functions to inhibit translation of new proteins. Specifically, ER stress causes oligomerization of the ER luminal domain (N-terminal) of PERK, which facilitates the trans-autophosphorylation of PERK's cytoplasmic kinase domain (C-terminal) at Thr-982. Thus, the phosphorylated form of PERK at the Thr 982 site is often assessed as a measure of ER stress.

Activated PERK phosphorylates eukaryotic translation initiation factor 2 alpha (eIF2 alpha), which inhibits translation of proteins to maintain homeostasis. Phospho-eIF2 alpha does not impact the translation of ATF4, however. Upon accumulation, ATF4 translocates to the nucleus, where it induces the expression of ER chaperones, autophagy/apoptosis genes (especially CHOP), oxidative response genes, and activates amino acid metabolism signaling pathways. PERK facilitates cell cycle exit during UPR, and is involved in the regulation of mitochondrial morphology as well as function.



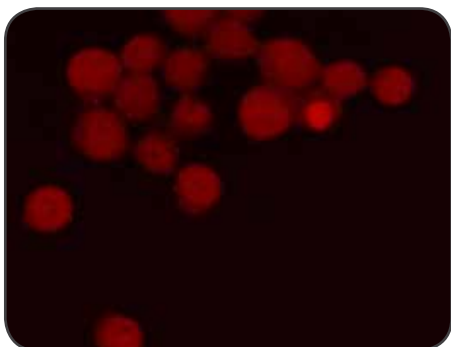
PERK Antibody [NBP1-78017]

WB analysis of lysates from PERK over-expressing 293T cells (lanes 1 & 2), Wild type MEFs (Lanes 3 & 4), and PERK knock-out MEFs (lanes 5 & 6). The MEFs were treated with vehicle (-) or Tunicamycin (+) for induction of ER stress.



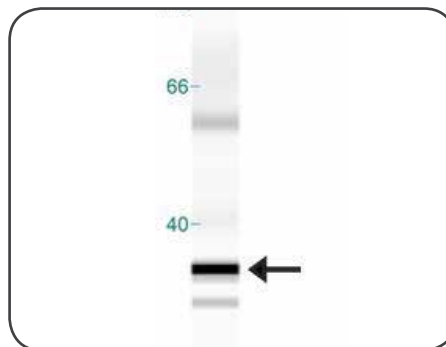
eIF2A Antibody (3A7B11) [NBP2-26296]

ICC/IF analysis of HeLa cells using eIF2A antibody with Dylight 488 (green) conjugated secondary antibody. Nuclei were counterstained with DAPI (blue). Alpha-tubulin was labelled using anti-tubulin with Dylight 550 (red) conjugated secondary antibody.



ATF4 Antibody (739441) [MAB7218]

ICC/IF analysis of Jurkat human acute T-cell leukemia cells using ATF4 mAb with NorthernLights™ 557-conjugated secondary antibody (red; Catalog # NL007). Specific staining was localized to nuclei and cytoplasm.



GADD153/CHOP Antibody (9C8) [NB600-1335]

Simple Western lane view shows a specific band for CHOP/GADD153 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

ER Stress / UPR Antibody Sampler Pack

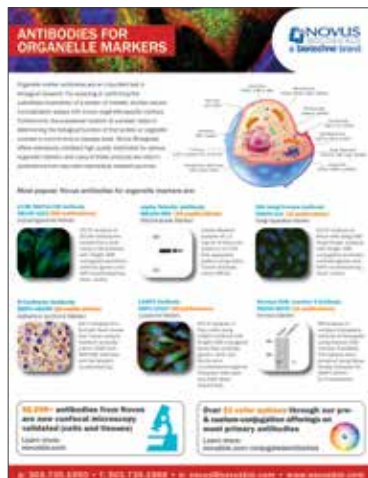
This pack contains sample size antibodies against the top six UPR markers: GRP78, pIRE1 alpha (Ser724), total IRE1 alpha, XBP1, ATF6, and CHOP. See catalog number: **NBP2-52746**

Related Publications

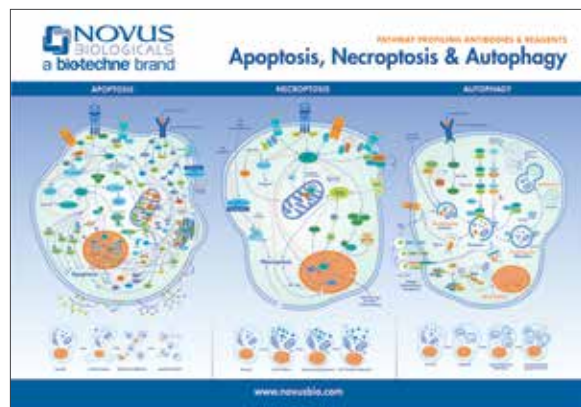
Western Blot Handbook



Organelle Markers Flyer



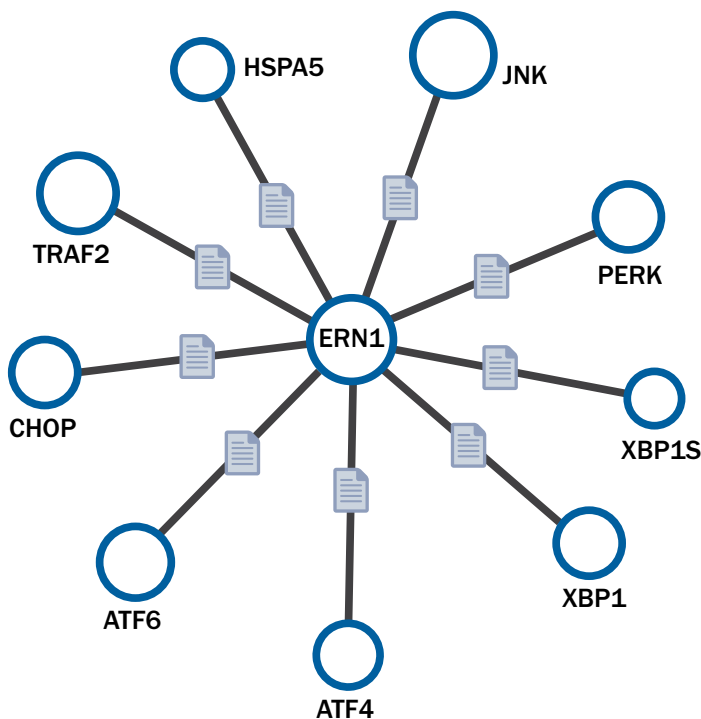
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