

Datasheet for 600-401-BZ6**IRE1p Antibody****Overview**

Description:	Anti-IRE1p (RABBIT) Antibody - 600-401-BZ6
Item No.:	600-401-BZ6
Size:	100 µg
Applications:	ELISA, IF, IHC, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background:	Accumulation of malformed proteins in the endoplasmic reticulum (ER) activates the unfolded protein response (UPR) and the upregulation of the ER molecular chaperones GRP78 and GRP 94. These proteins are normally bound to ER transmembrane proteins such as IRE1p and ATF6 but ER stress causes their dissociation. This allows IRE1p, a serine-threonine protein kinase to transduce the unfolded protein signal from the ER to the nucleus. IRE1p also has an endoribonuclease activity that is required to splice X-box binding protein (XBP1) mRNA converting it to a potent UPR transcriptional activation. Depletion of IRE1p through the expression of a dominant negative form of IRE1p has no effect on transfected cells, but cell death via apoptosis occurs under stress conditions that cause unfolded proteins to accumulate in the ER. Two alternatively spliced transcript variants encoding different isoforms have been found for this gene.
Synonyms:	IRE1p Antibody, IRE1, IRE1P, IRE1a, hIRE1p, IRE1, Endoplasmic reticulum-to-nucleus signaling 1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ERN1
Reactivity:	Human, Mouse, Rat
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-IRE1p antibody was prepared from whole rabbit serum produced by repeated immunizations with a 16 amino acid synthetic peptide from near the C-terminus of human IRE1p.
Purity/Specificity:	Anti-IRE1p Antibody was affinity purified from monospecific antiserum by immunoaffinity chromatography. Cross reactivity with IRE1p from other sources has not been determined.
Relevant Links:	• UniProtKB - O75460 • GeneID - 2081 • NCBI - O75460

Application Details

Tested Applications:	ELISA, IF, IHC, WB
Application Note:	Anti-IRE1p Antibody has been tested for use in ELISA, Western Blotting, Immunocytochemistry, Immunohistochemistry, and Immunofluorescence. Expect a band at approximately 110 kDa in Western Blots using positive control A-20 whole cell lysate, which can also be used in ICC and IF, or other specific cell lysates and tissues. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5000 - 1:20,000
IF:	2-20 µg/mL
IHC:	2 µg/mL
WB:	0.5-2 µg/mL

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1 mg/mL by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.02% (w/v) Sodium Azide
Stabilizer:	None

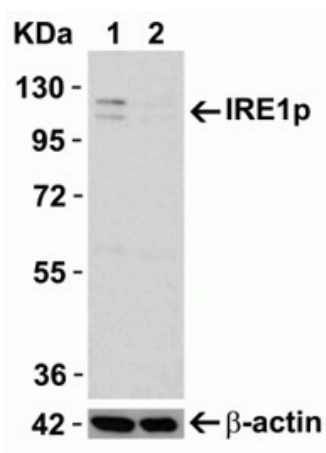
Shipping & Handling

Shipping Condition:	Dry Ice
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Storage Condition: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

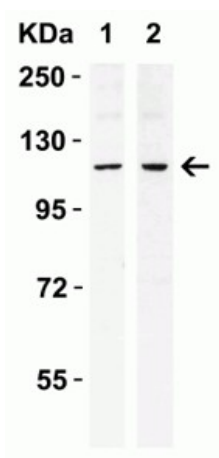
Expiration: Expiration date is one (1) year from date of receipt.

Images



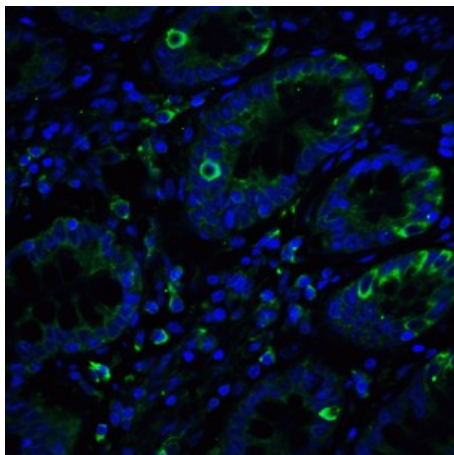
Western Blot

KO Validation in HeLa Cells. Loading: 10 µg of WT cell lysates (lane 1) or IRE1p KO cell lysates (lane 2). Antibodies: IRE1P (0.5 µg/mL) and beta-actin (1 µg/mL), 1h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



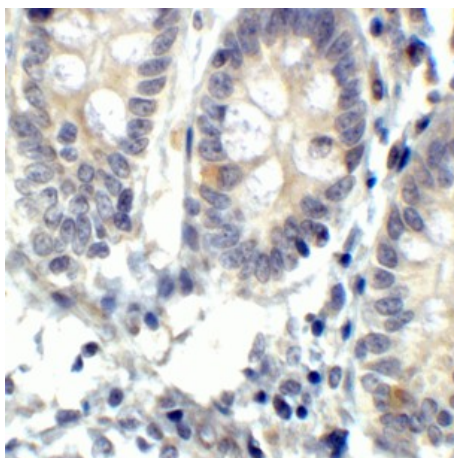
Western Blot

Western Blot Validation. Loading: 15 µg of human cell lysates per lane. Lane 1: Caco-2, Lane2: SK-N-SH. Antibodies: IRE1p (0.4 µg/mL), 1h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

**Immunofluorescence Microscopy**

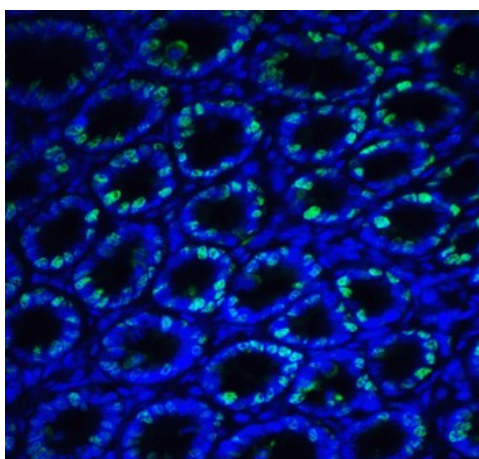
Immunofluorescence Validation of IRE1p.

Immunofluorescent analysis of 4% paraformaldehyde-fixed Human Small Intestine Tissue labeling IRE1p with at 20 $\mu\text{g/mL}$, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (green) and DAPI staining (blue).

**Immunohistochemistry**

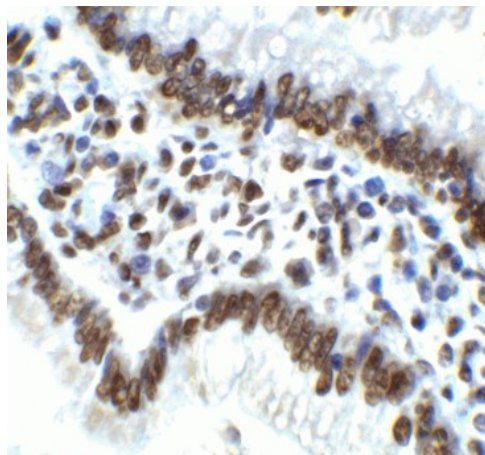
Immunohistochemistry Validation of IRE1p.

Immunohistochemical analysis of paraffin-embedded Human Small Intestine Tissue using anti-IRE1P antibody at 2 $\mu\text{g/mL}$. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter

**Immunofluorescence Microscopy**

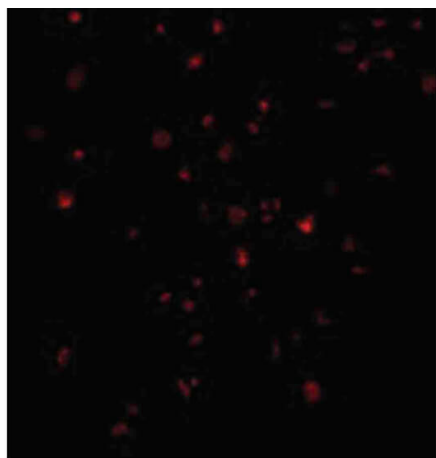
Immunofluorescence Validation of IRE1p.

Immunofluorescent analysis of 4% paraformaldehyde-fixed Rat Small Intestine Tissue labeling IRE1p at 20 $\mu\text{g/mL}$, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (green) and DAPI staining (blue).



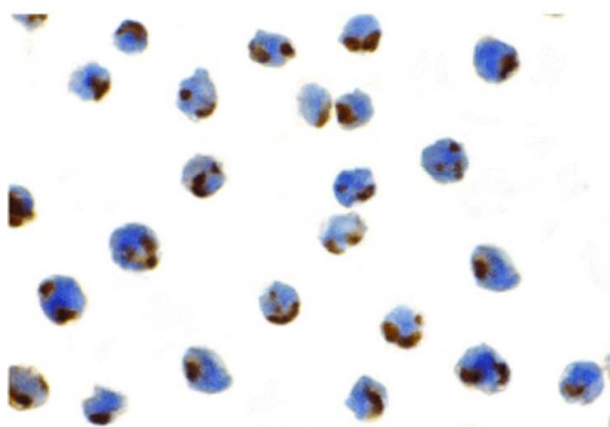
Immunohistochemistry

Immunohistochemistry Validation of IRE1p.
 Immunohistochemical analysis of paraffin-embedded Rat Small Intestine Tissue using anti-IRE1p antibody at 2 µg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.



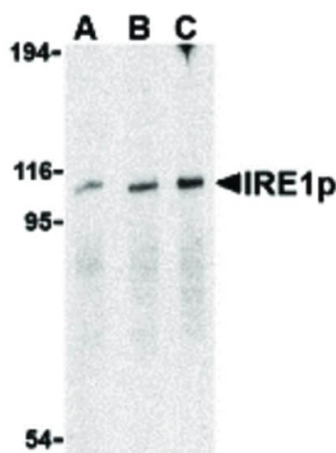
Immunofluorescence Microscopy

Immunofluorescence Microscopy of IRE1p antibody. Cell Type: A20 cells. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: IRE1p antibody at 2 µg/mL for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: IRE1p is located in the endoplasmic reticulum and cell membrane. Staining: IRE1p as red fluorescent signal.



Immunohistochemistry

Immunocytochemistry of IRE1p antibody. Cell Type: A-20 cells. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: IRE1p antibody at 1 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: IRE1p is located in the endoplasmic reticulum and cell membrane. Staining: IRE1p is stained brown with purple counterstain.



Western Blot

Western Blot of IRE1p antibody in A-20 cell lysate. Lane A: IRE1p antibody at 0.5 $\mu\text{g/mL}$. Lane B: IRE1p antibody at 1 $\mu\text{g/mL}$. Lane C: IRE1p antibody at 2 $\mu\text{g/mL}$. Load: 35 μg per lane. Primary antibody: IRE1p antibody at designated concentrations for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 110 kDa, 114 kDa for IRE1p. Other band(s): IRE1p splice variants and isoforms.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.