Product Datasheet

IRE1 alpha Antibody - BSA Free
NB100-2324

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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Reviews: 2 Publications: 62

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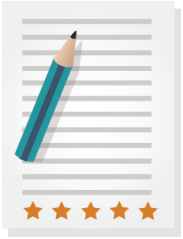
Updated 2/21/2025 v.20.1



NB100-2324

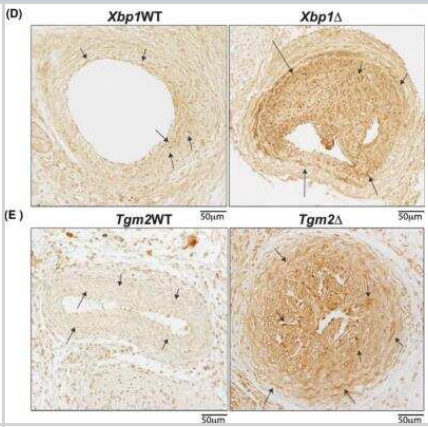
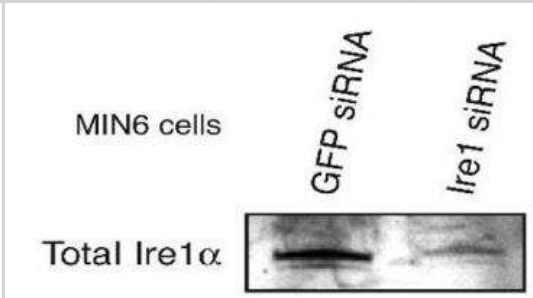
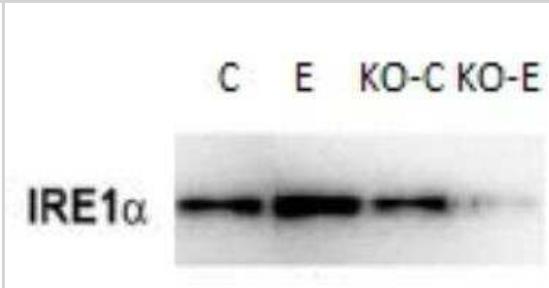
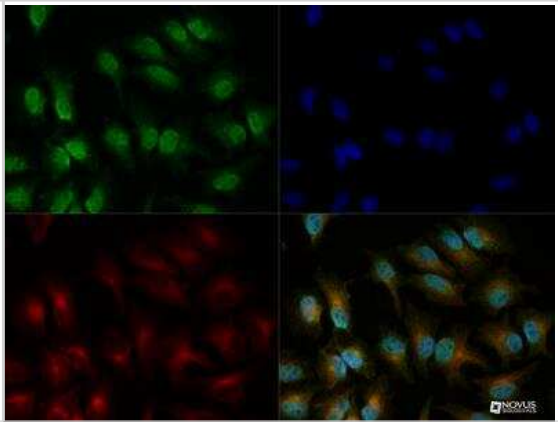
IRE1 alpha Antibody - BSA Free

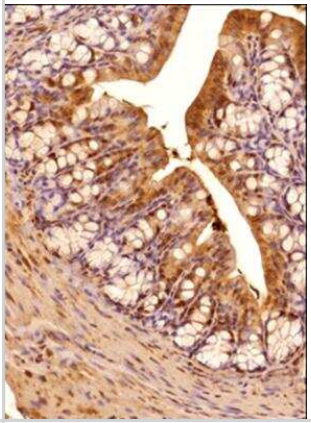
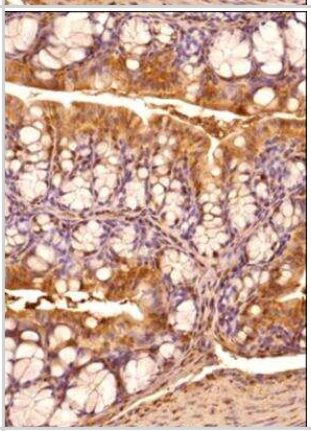
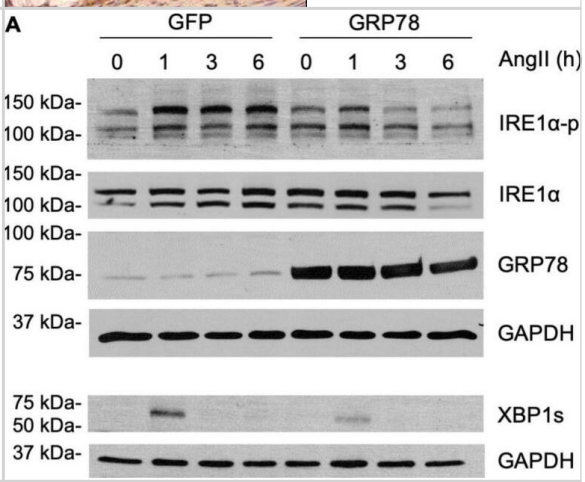
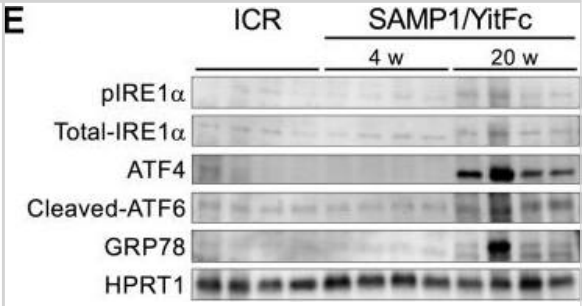
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	110 kDa

Product Description	
Description	NB100-2324 is useful for the detection of endogenous total IRE1 Alpha protein. For detecting phospho-IRE1 Alpha (Ser-724), we suggest NB100-2323 and it is recommended to normalize phospho-IRE1 alpha band intensity/immunoreactivity with total-IRE1 alpha using NB100-2324 or NB110-59971.
Host	Rabbit
Gene ID	2081
Gene Symbol	ERN1
Species	Human, Mouse, Rat
Immunogen	This IRE1 alpha antibody was raised against a synthetic peptide within the human IRE1 alpha protein (within residues 700-800). [Swiss-Prot #O75460]

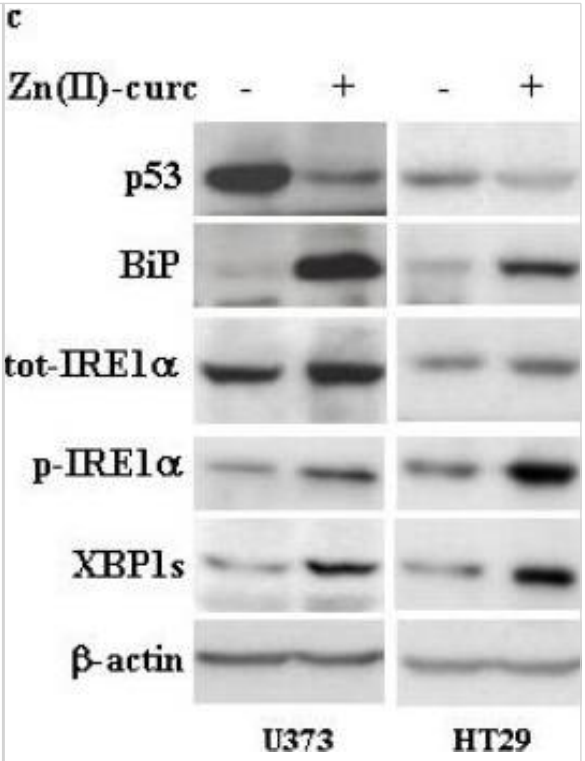
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000 - 1:2000, Immunohistochemistry 1:250 - 1:500, Immunocytochemistry/ Immunofluorescence 1:100 - 1:250, Immunohistochemistry-Paraffin 1:250 - 1:500, Knockout Validated reported in scientific literature (PMID 31159306), Knockdown Validated
Application Notes	NB100-2324 is useful for the detection of endogenous total IRE1 Alpha protein. For detecting phospho-IRE1 Alpha (Ser-724), we suggest NB100-2323 and it is recommended to normalize phospho-IRE1 alpha band intensity/immunoreactivity with total-IRE1 alpha using NB100-2324 or NB110-59971. In Western blot a band at ~110 kDa is observed. In ICC/IF endoplasmic reticulum staining was observed in HeLa cells. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Immunohistochemistry: IRE1 alpha Antibody [NB100-2324] - XBP1 and TG2 deficiency promote increased IRE1a protein levels in cultured VSMCs and in neointima in situ. (D) Xbp1WT and Xbp1 delta WT and (E) Tgm2 WT and Tgm2 delta carotid artery sections were analyzed by IHC for total IRE1a. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0212235) licensed under a CC-BY license.	
Western Blot: IRE1 alpha Antibody [NB100-2324] - Total IRE1 Alpha protein in lysates from Min6 cells which were transfected with GFP-siRNA or Ire1-siRNA. Theoretical molecular weight: 110 kDa.	
Western Blot: IRE1 alpha Antibody [NB100-2324] - ALOX15 knockout blocks alcohol-induced up-regulation of IRE1a in mouse liver. Image from verified customer review.	
Immunocytochemistry/Immunofluorescence: IRE1 alpha Antibody [NB100-2324] - IRE-1 alpha antibody was tested in HeLa cells at 1:200 against Dylight 488 (Green). Alpha tubulin and nuclei were counterstained against Dylight 568 (Red) and DAPI (Blue).	

Immunohistochemistry-Paraffin: IRE1 alpha Antibody [NB100-2324] - Analysis of a formalin fixed mouse colon tissue section using total IRE1 alpha antibody (NB100-2324) at 1:300. Note a strong cytoplasmic signal in various cells of colonic villi (columnar epithelial and goblet cells) and the muscular layer. A few cells, potentially with active UPR, showed nuclear positivity also while the signal was negligible in cells at base of crypts and lamina propria.	
Immunohistochemistry-Paraffin: IRE1 alpha Antibody [NB100-2324] - Analysis of a formalin fixed mouse colon tissue section using total IRE1 alpha antibody (NB100-2324) at 1:300. Note a strong cytoplasmic signal in various cells of colonic villi (columnar epithelial and goblet cells) and the muscular layer. Some cells, most likely with active UPR, depicted nuclear positivity also while the signal was negligible in crypts cells and lamina propria.	
The inositol-requiring enzyme 1 α (IRE1α)/ X-box-binding-protein 1 spliced isoform (XBP1s) arm of unfolded protein response (UPR) is induced by angiotensin II in VSMCs. (A–C) The rat aortic VSMCs infected with adenovirus encoding GRP78 or control GFP (100 moi) for 48 h were stimulated with 100 nM AngII (AII) for 1–6 h & immunoblotting was performed as indicated. (A) Representative blots from 4 independent experiments. (B) Signal intensity was used to calculate the expression ratio of XBP1s to GAPDH. (C) Signal intensity was used to calculate the IRE1α Ser724 phosphorylation ratio to the total IRE1α. The bars in the graphs show the mean ± SD from 4 independent experiments. * indicates $p < 0.05$. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32679678), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Abnormal Paneth cells show ER stress.(A, B) Representative transmission electron microscopy images of Paneth cells at the base of ileal crypts in (A) ICR & (B) SAMP1/YitFc mice. Scale bars indicate 2 μm. (C, D) Quantitative analysis of (C) granule number & (D) ER lumen diameter in Paneth cells (n = 3/each week for SAMP1/YitFc mice). For the measurements, three Paneth cells were randomly selected from each mouse. (E) SDS–PAGE Western blot analysis of ER stress markers, pIRE1α, ATF4, cleaved-ATF6, & GRP78 in ileal crypts (n = 4/each group). Total-IRE1α & HPRT1 was used as loading control. (F) Relative expression level of ER stress markers calculated from the band intensity. Error bars represent mean ± SEM. (C, D, F) Statistical significance was evaluated by t test in (C, D), & one-way ANOVA followed by Tukey's post hoc test in (F). $P < 0.05$ was considered statistically significant. *$P < 0.05$, †$P < 0.01$, §$P < 0.001$. E, ER; G, granules; N, nucleus; n.s., not significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32345659), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: IRE1 alpha Antibody [NB100-2324] - Zn (II)-curc induces endoplasmic reticulum (ER) stress in mutant p53H273-carrying cells. (a) Representative photomicrographs of ER-Red Fluorescence staining in U373 cells untreated (Mock) or treated with Zn (II)-curc (100 µg/mL) for 16 h (Original magnification: 40×). (b) Quantization of ER content in U373 cells from ER-Red Fluorescence-stained cells. Mean fluorescence intensity (MFI) of each individual cell was normalized to cell size & expressed as fold-change compared with untreated cells at the same time point. Histograms represent the mean ± SD of three independent experiments. * p ≤ 0.05. (c) Western blot analysis of p53, BiP, total (tot) IRE1α, phosphorylated (p) IRE1α, & XBP1 spliced (s) protein levels evaluated in U373 & HT29 cells untreated or treated with Zn (II)-curc (100 µg/mL) for 24 h. (d) Densitometric analysis was performed using Image J software to calculate the ratio of the protein levels, as detected in (c), vs. β-actin. Histograms represent the mean ± SD of three independent experiments. * p ≤ 0.05. (e) Total mRNA was extracted from U373 & HT29 cells untreated or treated with Zn (II)-curc (100 µg/mL) for 24 h. Spliced (s) Xbp1 gene expression was assayed by the polymerase chain reaction (PCR) of reverse-transcribed cDNA. Densitometric analysis was performed using Image J software to calculate the Xbp1s/28S ratio. Histograms represent the mean ± SD of three independent experiments. * p ≤ 0.05. (f) p53 gene expression was assayed by PCR as in (e). The p53/28S ratio is indicated. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32138264), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Y Ohtake, K Matsuhisa, M Kaneko, S Kanemoto, R Asada, K Imaizumi, A Saito Axonal Activation of the Unfolded Protein Response Promotes Axonal Regeneration Following Peripheral Nerve Injury Neuroscience, 2018-02-10;0(0):. 2018-02-10 [PMID: 29438804]

Hacisuleyman E, Hale CR, Noble N et Al. Neuronal activity rapidly reprograms dendritic translation via eIF4G2:uORF binding Nat Neurosci 2024-04-08 [PMID: 38589584]

Moellmann J, Krueger K, Wong DWL et al. 2,8-Dihydroxyadenine-induced nephropathy causes hexosylceramide accumulation with increased mTOR signaling, reduced levels of protective SirT3 expression and impaired renal mitochondrial function Biochimica et biophysica acta. Molecular basis of disease 2023-08-01 [PMID: 37536502]

Shimizu Y, Nakamura K, Yoshii A et al. Paneth cell alpha-defensin misfolding correlates with dysbiosis and ileitis in Crohn\'s disease model mice Life Sci Alliance 2020-06-01 [PMID: 32345659]

M Oro?, M Grochowski, A Jaiswar, J Legierska, K Jastrz?bsk, M Nowak-Niez, M Ko?os, W Ka?miercza, T Olesi?ski, M Lenarcik, M Cybulska, M Mikula, A ?ylicz, M Mi?czy?ska, K Zettl, JR Wi?niewski, D Walerych The molecular network of the proteasome machinery inhibition response is orchestrated by HSP70, revealing vulnerabilities in cancer cells Cell Reports, 2022-09-27;40(13):111428. 2022-09-27 [PMID: 36170818]

Da L, Jeong P, Yu L et al. PERK prevents hepatic lipotoxicity by activating the p62-ULK1 axis-mediated noncanonical KEAP1-Nrf2 pathway. Redox Biol. 2022-01-14 [PMID: 35091323]

Wells KM, He K, Pandey A et al. Brucella activates the host RIDD pathway to subvert BLOS1-directed immune defense eLife 2022-05-19 [PMID: 35587649]

Liao Y, Huang J, Wang Z et al. The phosphokinase activity of IRE1? prevents the oxidative stress injury through miR-25/Nox4 pathway after ICH CNS neuroscience & therapeutics 2023-11-23 [PMID: 37994671] (WB, Mouse)

Delmotte P, Yap J, Dasgupta D, Sieck G Chemical Chaperone 4-PBA Mitigates Tumor Necrosis Factor Alpha-Induced Endoplasmic Reticulum Stress in Human Airway Smooth Muscle International Journal of Molecular Sciences 2023-10-31 [PMID: 37958799] (WB, Human)

Details:
1:1000 WB dilution

Wang X, Xin H, Zhang C et al. GRP94 Inhabits the Immortalized Porcine Hepatic Stellate Cells Apoptosis under Endoplasmic Reticulum Stress through Modulating the Expression of IGF-1 and Ubiquitin International Journal of Molecular Sciences 2022-11-14 [PMID: 36430538] (Western Blot)

Kim SM, Han Y, Yu SM, Kim SJ. Gallotannin attenuates 2?deoxy?D?glucose?induced dedifferentiation and endoplasmic reticulum stress through inhibition of inositol?requiring enzyme 1 downstream p38 kinase pathway in chondrocytes Molecular Medicine Reports 2019-10-25 [PMID: 31661132]

Torrens JN, Hetzer SM, Evanson NK. Brief Oxygen Exposure after Traumatic Brain Injury Hastens Recovery and Promotes Adaptive Chronic Endoplasmic Reticulum Stress Responses International Journal of Molecular Sciences 2023-06-06 [PMID: 37372978]

More publications at http://www.novusbio.com/NB100-2324

Procedures

Immunocytochemistry/Immunofluorescence protocol for IRE1 alpha Antibody (NB100-2324)
Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4 degrees C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

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Products Related to NB100-2324

NB820-59244	Human Pancreas Whole Tissue Lysate (Adult Whole Normal)
NB100-2324PEP	IRE1 alpha Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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