Product Datasheet

Calreticulin Antibody - BSA Free
NB600-101

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Publications: 28

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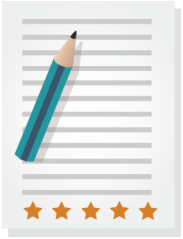
Updated 2/17/2025 v.20.1



NB600-101

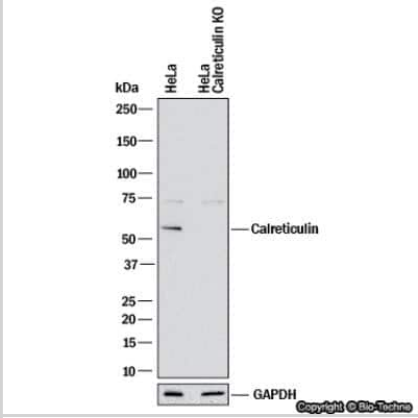
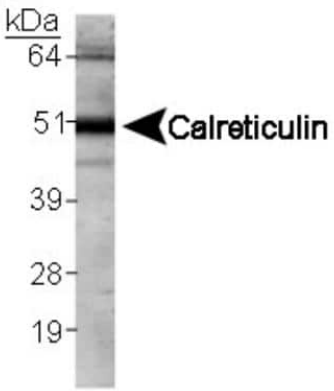
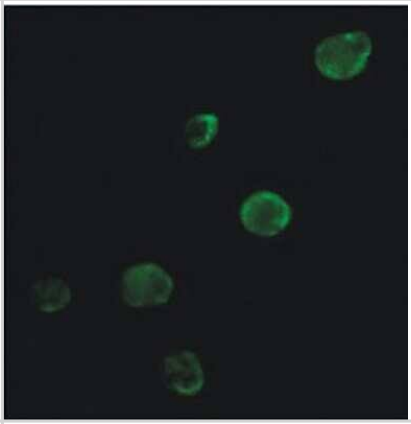
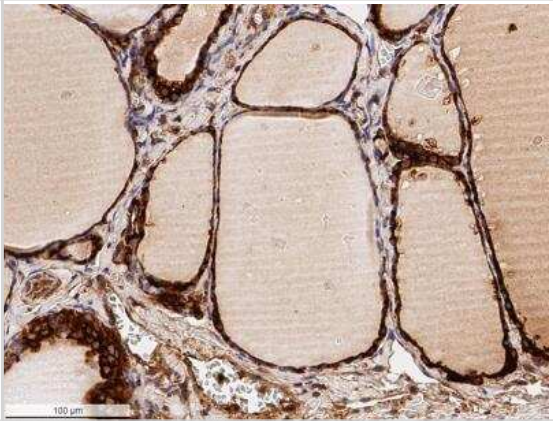
Calreticulin Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	48 kDa

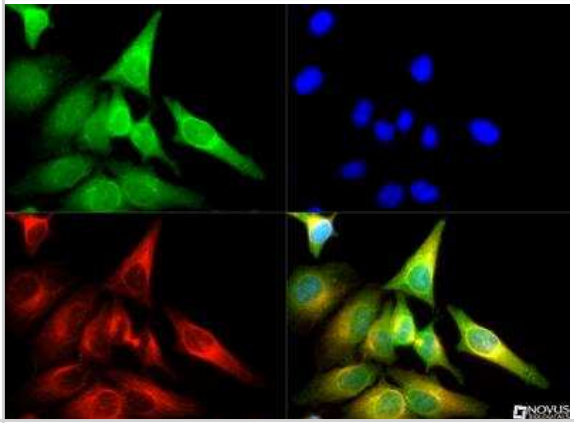
Product Description	
Host	Rabbit
Gene ID	811
Gene Symbol	CALR
Species	Human, Mouse, Rat, Bovine, Hamster, Primate
Reactivity Notes	Other species not tested.
Marker	Endoplasmic Reticulum Marker
Immunogen	Calreticulin Antibody was developed against a fusion protein to mouse Calreticulin [Uniprot: P14211]

Product Application Details	
Applications	Western Blot, Simple Western, Dot Blot, Electron Microscopy, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Protein Array, Block/Neutralize, Knockdown Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:50, Flow Cytometry reported in scientific literature (PMID 19171874), Immunohistochemistry 1:50 - 1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:250, Immunoprecipitation reported in scientific literature (PMID 15161937), Immunohistochemistry-Paraffin 1:50 -1:200, Dot Blot, Electron Microscopy, Protein Array reported in scientific literature (PMID 17897946), Knockdown Validated 1:1500, Block/Neutralize
Application Notes	In Western blot, a band is observed at ~55 kDa. 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. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 64 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

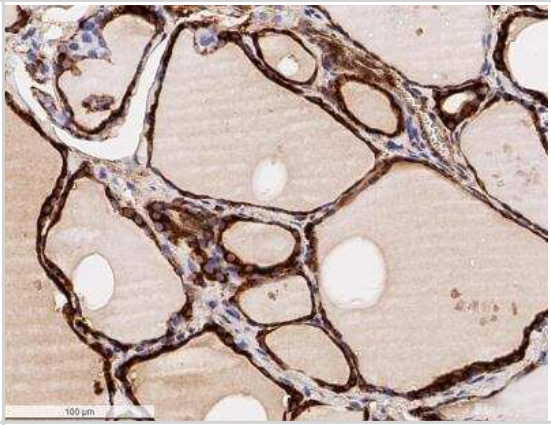
Images

Western Blot: Calreticulin Antibody [NB600-101] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Calreticulin knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1500 of Rabbit Anti-Human Calreticulin Polyclonal Antibody (Catalog # NB600-101) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for Calreticulin at approximately 55 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.	
Western Blot: Calreticulin Antibody [NB600-101] - Human kidney lysate.	
Immunocytochemistry/Immunofluorescence: Calreticulin Antibody [NB600-101] - Immunofluorescence staining of Calreticulin in HCT15 colon cancer cells using NB600-101. Secondary antibody was conjugated with Alexa Fluor 488. Photo courtesy of Dr. Birkenkamp Demtroeder, Arhus University Hospital.	
Immunohistochemistry-Paraffin: Calreticulin Antibody [NB600-101] - Analysis of a FFPE tissue section of human thyroid gland using 1:50 dilution of Calreticulin antibody. The signal was developed using HRP-DAB based detection method which followed counterstaining of the nuclei with hematoxylin. This representative section shows a strong positivity of Calreticulin in the follicular epithelial cells, wherein the signal was found to be very intense in the perinuclear region of the cells which correlates well with Endoplasmic reticulum localization of this protein. The para-follicular cells, endothelial cells of blood vessels (not the RBCs though) and the loose connective tissue in the section showed a weak cytoplasmic staining. Some staining was observed in the follicles/colloids also which is potentially the secreted form of Calreticulin.	

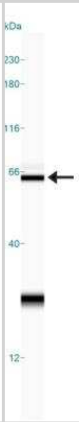
Immunocytochemistry/Immunofluorescence: Calreticulin Antibody [NB600-101] - The Calreticulin antibody NB600-101 was tested in HeLa cells at a 1:250 dilution against DyLight 488 (Green). Alpha-tubulin and nuclei were counterstained against DyLight 550 (Red) and DAPI (Blue), respectively.



Immunohistochemistry-Paraffin: Calreticulin Antibody [NB600-101] - Analysis of a FFPE tissue section of human thyroid gland using 1:50 dilution of Calreticulin antibody. The signal was developed using HRP-DAB based detection method which followed counterstaining of the nuclei with hematoxylin. This representative section shows a strong positivity of Calreticulin in the follicular epithelial cells, wherein the signal was found to be very intense in the perinuclear region of the cells which correlates well with Endoplasmic reticulum localization of this protein. The para-follicular cells, endothelial cells and the loose connective tissue in the section showed a weak cytoplasmic staining. Some staining was observed in the follicles/colloids also which is potentially the secreted form of Calreticulin.



Simple Western: Calreticulin Antibody [NB600-101] - Lane view shows a specific band for Calreticulin in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Calreticulin was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-Calreticulin Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB600-101AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 40X objective.

Publications

Sanchez-Herrero E, Campos-Silva C, Caceres-Martell Y et al. ALK-Fusion Transcripts Can Be Detected in Extracellular Vesicles (EVs) from Nonsmall Cell Lung Cancer Cell Lines and Patient Plasma: Toward EV-Based Noninvasive Testing Clinical chemistry [PMID: 35348673] (ICC/IF, Human)

Zheng C, Sui B, Zhang X et al. Apoptotic vesicles restore liver macrophage homeostasis to counteract type 2 diabetes Journal of Extracellular Vesicles 2021-05-01 [PMID: 34084287] (Human)

Harada K, Matsuoka H, Toyohira Y et al. Mechanisms for establishment of GABA signaling in adrenal medullary chromaffin cells Journal of neurochemistry 2021-03-11 [PMID: 33704788]

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Roberti MP, Yonekura S, Duong CPM et al. Chemotherapy-induced ileal crypt apoptosis and the ileal microbiome shape immunosurveillance and prognosis of proximal colon cancer Nat. Med. 2020-05-25 [PMID: 32451498] (Mouse)

Musante L, Bontha Sv, La Salvia S et Al. Rigorous characterization of urinary extracellular vesicles (uEVs) in the low centrifugation pellet - a neglected source for uEVs Sci Rep Feb 28 2020 12:00AM [PMID: 32111925] (WB, Human)

Lekszas C, Foresti O, Raote I et Al. Biallelic TANGO1 mutations cause a novel syndromal disease due to hampered cellular collagen secretion Elife 2020-02-26 [PMID: 32101163] (ICC/IF, Human)

Dong X, Wu W, Ma L et al. Collectin-11 Is an Important Modulator of Retinal Pigment Epithelial Cell Phagocytosis and Cytokine Production J Innate Immun 2017-08-04 [PMID: 28772263] (FLOW, Mouse)

Yamada HY, Kumar G, Zhang Y et al. Systemic chromosome instability in Shugoshin-1 mice resulted in compromised glutathione pathway, activation of Wnt signaling and defects in immune system in the lung. Oncogenesis. 2016-08-16 [PMID: 27526110] (WB, IF/IHC)

Bazwinsky-Wutschke I, Wolgast S, Muhlbauer E, Peschke E. Distribution patterns of calcium-binding proteins in pancreatic tissue of non-diabetic as well as type 2 diabetic rats and in rat insulinoma beta-cells (INS-1). Histochem Cell Biol. 2010-07-07 [PMID: 20607274] (Rat)

Zhou M, He HJ, Suzuki R et al. Localization of sulfonylurea receptor subunits, SUR2A and SUR2B, in rat heart. J Histochem Cytochem 2007-08-01 [PMID: 17438353] (Rat)

Teng CY, van Oers MM, Wu TY. Additive effect of calreticulin and translation initiation factor eIF4E on secreted protein production in the baculovirus expression system. Appl Microbiol Biotechnol. 2013-10-01 [PMID: 23900798] (WB)

More publications at http://www.novusbio.com/NB600-101

Procedures

Western Blot protocol for Calreticulin Antibody (NB600-101)

Western Blot Procedure

1) Scrape cells* off culture dishes and centrifuge.
2) Dissolve cell pellet in decanoyl-N-methyl glucamide (MEGA-10)** and clarify by centrifugation.
3) Mix 30 mg of protein*** with sample buffer containing mercaptoethanol and SDS and run on a 10% SDS gel. The protein was electroblotted on to nitrocellulose.
4) Block nitrocellulose with 5% powdered milk in PBS for 1 hour.
5) Wash the blot with PBS.
6) Add the antibody at a concentration of 1:1000 in 5% powdered milk/PBS and incubate for 1 hour.
7) Wash 3 x 5 minutes with PBS.
8) Add peroxidase-labelled anti-rabbit second antibody in PBS at a concentration of 1:3000 and shake for 1 hour.
9) Wash extensively with PBS.
10) Develop with ECL reagents (Amersham). For this experiment, the film was exposed to the blot for 10 seconds.
****

i. The cells used were the H4IIE rat hepatoma cell line and the NBL-1 bovine renal epithelial cell line.
ii. The detergent used is not critical. MEGA-10 has the advantage of not interfering with the Bradford protein reagent.
iii. Whole cells were used in this experiment. If 30mg of a cell membrane fraction were used a more intense band would be seen.
iv. In this experiment, the antibody was used at 1:1000, but since whole cell protein was used and only 10 seconds development was required it could presumably be used at a lower concentration for many applications.

Immunohistochemistry protocol for Calreticulin Antibody (NB600-101)

Immunohistochemistry Procedure

1) Paraffin-embedded sections were treated using an HIER [heat-induced epitope retrieval] protocol.
2) NB 600-101 was diluted 1:50 with a DAKO antibody diluent
3) Sections and primary antibody were incubated at RT for 30 minutes.
4) Sections were incubated with a DAKO secondary, as per manufacturer'??s protocol.
5) Sections were then stained with DAB and H&E.
6) Sections were mounted with Faramount solution.
7) Result of staining was strong.

**NOTE: normal colon mucosa and adenocarcinoma tissues were used as positive controls for this antibody.

Immunocytochemistry/Immunofluorescence protocol for Calreticulin Antibody (NB600-101)

Immunofluorescence Procedure

1) Cell cultures were treated using an HIER [heat-induced epitope retrieval] protocol.
2) Calreticulin polyclonal antibody [NB 600-101] was diluted 1:50 with a DAKO antibody diluent
3) Cultures and primary antibody were incubated at RT for 30 minutes.
4) Cultures were incubated with an Alexa green fluorescent secondary, as per manufacturer'??s protocol.
5) Cultures were observed on a Zeiss Axioconvert microscope and with DigiPix software.

**NOTE: COS7 and HCT29 cell cultures were used as positive controls for this antibody.

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Products Related to NB600-101

NBL1-08655	Calreticulin Overexpression Lysate
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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