# **Product Datasheet**

# Calreticulin Antibody NB600-103

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.



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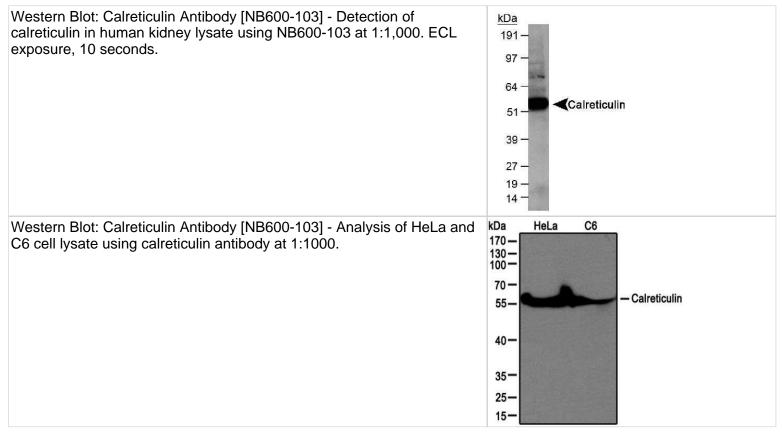
## NB600-103

Calreticulin Antibody

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Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera
Target Molecular Weight	48 kDa
Product Description	
Host	Rabbit
Gene Symbol	CALR
Species	Human, Mouse
Marker	Endoplasmic Reticulum Marker
Immunogen	Calreticulin Antibody was developed against a full-length protein to mouse Calreticulin [UniProt# P14211]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry- Paraffin, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000-1:10000, Simple Western 1:500, Immunohistochemistry 1:20, Immunocytochemistry/ Immunofluorescence 1:20-1:100. Use reported by customer review, Immunohistochemistry-Paraffin 1:20, Immunohistochemistry- Frozen reported in scientific literature (PMID 26643212), Knockout Validated, Knockdown Validated 1:2500
Application Notes	A band at approx. 55 kDa is seen in Western Blot. Note that Calreticulin has several reported isoforms that range from 48-62 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.



Images Knockout Validated: Calreticulin Antibody [NB600-103] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Calreticulin knockout (KO) HeLa cell line. PVDF membrane was HeLa HeLa kDa probed with 1:2500 of Rabbit Anti-Human Calreticulin Polyclonal 250 Antibody (Catalog # NB600-103) followed by HRP-conjugated Anti-150-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was 75 detected for Calreticulin at approximately 55 kDa (as indicated) in the 50 parental HeLa cell line, but is not detectable in the knockout HeLa cell 37 line. This experiment was conducted under reducing conditions. 25 20 15 Simple Western: Calreticulin Antibody [NB600-103] - Lane view shows a specific band for Calreticulin in 0.2 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Immunocytochemistry/Immunofluorescence: Calreticulin Antibody [NB600-103] - IF Confocal analysis of 3T3 cells using Calreticulin antibody (NB600-103, 1:20). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue). Immunohistochemistry-Paraffin: Calreticulin Antibody [NB600-103] -Analysis of FFPE human prostate cancer using Calreticulin antibody at 1:20 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Cytoplasmic staining in glands was observed. Staining was performed by Histowiz.



#### **Publications**

Xie YX, Naseri NN, Fels J et al. Lysosomal exocytosis releases pathogenic alpha-synuclein species from neurons in synucleinopathy models Nature communications 2022-08-22 [PMID: 35995799] (WB, Mouse)

Lou J, Aragaki M, Bernards N Et al. Repeated porphyrin lipoprotein-based photodynamic therapy controls distant disease in mouse mesothelioma via the abscopal effect Nanophotonics 2021-08-02 [PMID: 36405502] (IHC-P, Mouse)

Soltic D, Bowerman M, Stock J et al. Multi-Study Proteomic and Bioinformatic Identification of Molecular Overlap between Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) Brain Sci 2018-12-04 [PMID: 30518112] (WB, Mouse)

Chavez-Valdez R, Flock DL, Martin LJ, Northington FJ. Endoplasmic reticulum pathology and stress response in neurons precede programmed necrosis after neonatal hypoxia-ischemia. Int. J. Dev. Neurosci. 2015-11-28 [PMID: 26643212] (IHC-Fr, Mouse)

Varjak M, Saul S, Arike L et al. Magnetic Fractionation and Proteomic Dissection of Cellular Organelles Occupied by the Late Replication Complexes of Semliki Forest Virus. J Virol 2013-09-01 [PMID: 23864636] (WB, Human)

Leslie EM et al. Differential inhibition of rat and human Na+-dependent taurocholate cotransporting polypeptide (NTCP/SLC10A1)by bosentan: a mechanism for species differences in hepatotoxicity. J Pharmacol Exp Ther321 (3):1170-8. 2007-06-01 [PMID: 17374746]

Nurden P et al. Impaired megakaryocytopoiesis in type 2B von Willebrand disease with severe thrombocytopenia. Blood108(8):2587-95. 2006-10-15 [PMID: 16720832] (WB, Human)



#### **Procedures**

#### Serum protocol for Calreticulin Antibody (NB600-103)

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in TBS for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 1 hour at room temperature.

6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

7. Dilute the rabbit anti-calreticulin primary antibody (NB 600-103) in blocking buffer and incubate 1.5 hours at room temperature.

8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).

11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Pierce's ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.





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# Products Related to NB600-103

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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