Product Datasheet

Calreticulin Antibody (1G6A7) - BSA Free
NBP1-47518

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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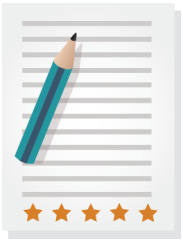
Updated 10/23/2024 v.20.1



NBP1-47518

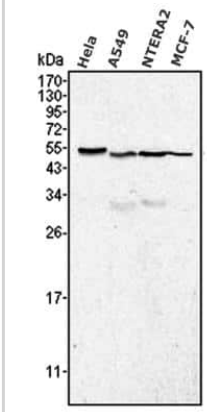
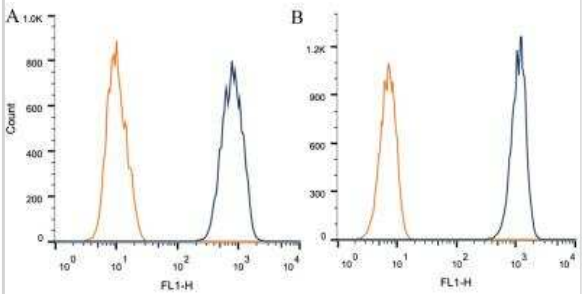
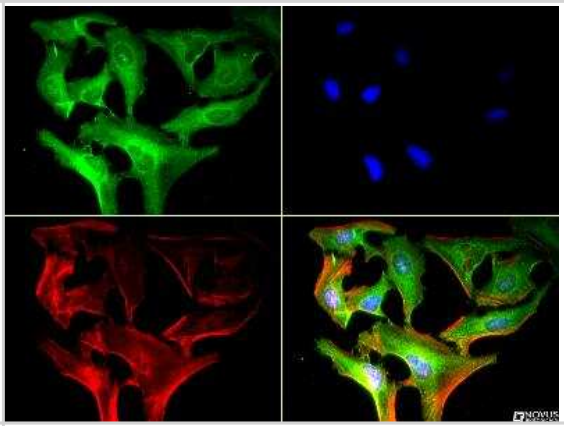
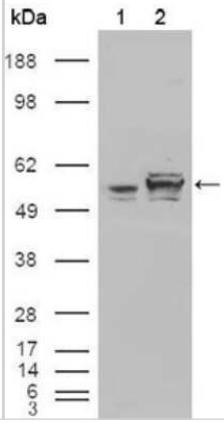
Calreticulin Antibody (1G6A7) - BSA Free

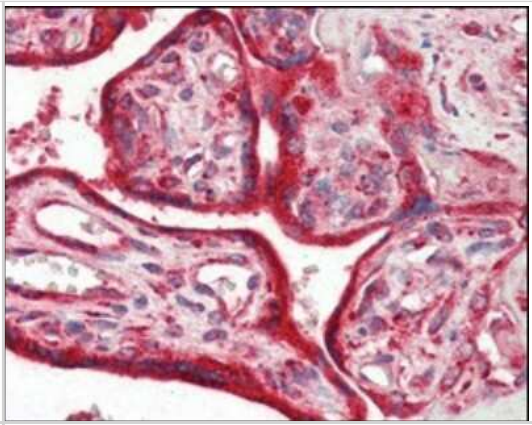
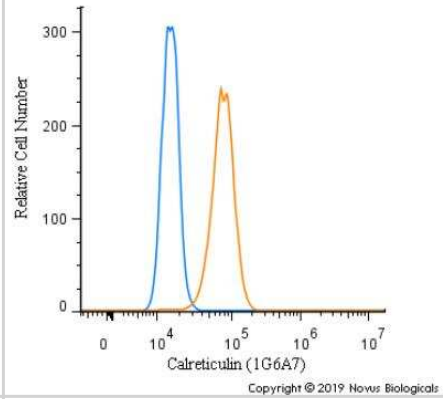
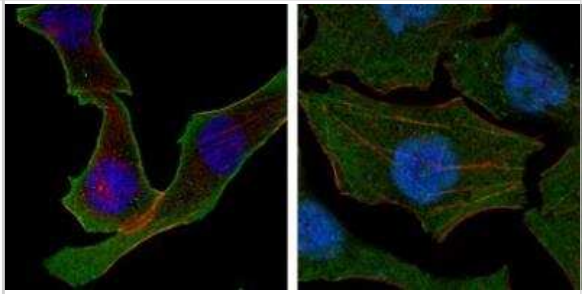
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1G6A7
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Ammonium sulfate precipitation
Buffer	PBS
Target Molecular Weight	48 kDa

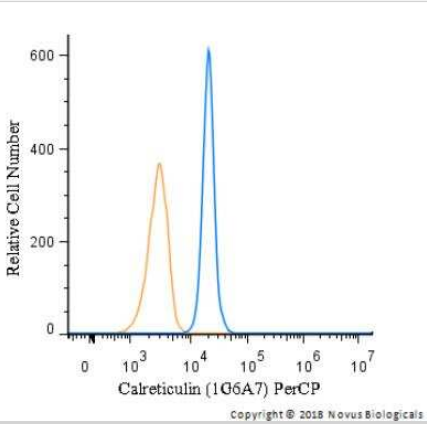
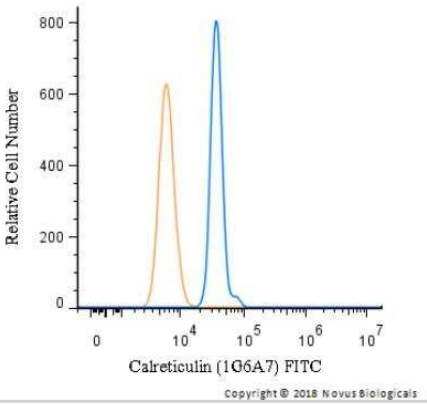
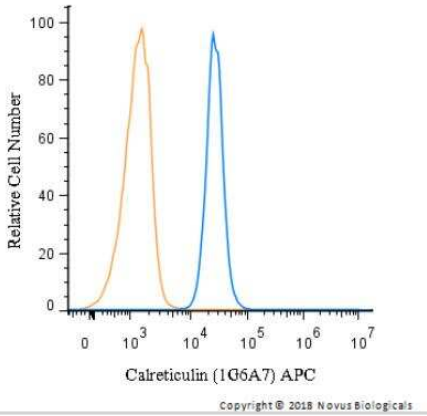
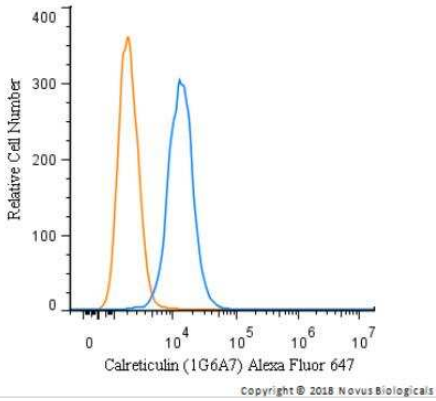
Product Description	
Host	Mouse
Gene ID	811
Gene Symbol	CALR
Species	Human, Mouse
Marker	Endoplasmic Reticulum Marker
Immunogen	Calreticulin Antibody (1G6A7) was developed against a synthetic peptide corresponding to the C-terminus (EEEDVPGQAKDELC) of human Calreticulin, conjugated to KLH. [UniProt# P27797]

Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, In vitro assay
Recommended Dilutions	Western Blot 1:500 - 1:2000, Flow Cytometry 1 ug/ml, ELISA 1:1000, Immunohistochemistry 1:200 - 1:1000, Immunocytochemistry/ Immunofluorescence 1:200 - 1:1000, Immunohistochemistry-Paraffin 1:200 - 1:1000, In vitro assay reported in scientific literature (PMID 33114476), Flow (Intracellular) 1 ug/ml
Application Notes	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.

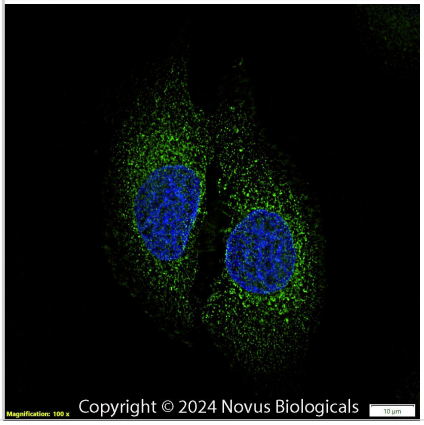
Images

Western Blot: Calreticulin Antibody (1G6A7) [NBP1-47518] - Analysis of whole cell lysates from HeLa, A549, NTERA2 and MCF-7 using Calreticulin antibody clone 1G6A7. The antibody generated a specific band of Calreticulin protein at ~48 kDa molecular weight position.	
Flow (Intracellular): Calreticulin Antibody (1G6A7) [NBP1-47518] - Intracellular staining of 1 x 10^6 CHO (A) and HEK-293 (B) cells using Calreticulin antibody (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10^6 cells was used.	
Immunocytochemistry/Immunofluorescence: Calreticulin Antibody (1G6A7) [NBP1-47518] - Antibody was tested in HeLa cells at a 1:250 against DyLight 488 (Green). Actin was counterstained against Phalloidin 568 (Red) and cells were mounted in DAPI Flouromount (Blue).	
Western Blot: Calreticulin Antibody (1G6A7) [NBP1-47518] - Analysis using Calreticulin mouse mAb against HEK293T cells transfected with the pCMV6-ENTRY control (1) and pCMV6-ENTRY Calreticulin cDNA (2).	

Immunohistochemistry-Paraffin: Calreticulin Antibody (1G6A7) [NBP1-47518] - Analysis of paraffin-embedded human placenta tissues using anti-Calreticulin mAb.	
Flow Cytometry: Calreticulin Antibody (1G6A7) [NBP1-47518] - An intracellular stain was performed on A431 cells with Calreticulin (1G6A7) Antibody NBP1-47518 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 488.	
Immunocytochemistry/Immunofluorescence: Calreticulin Antibody (1G6A7) [NBP1-47518] - Analysis of 3T3-L1 cells using anti-Calreticulin mAb (green). DRAQ5 fluorescent DNA dye (blue).	
Immunocytochemistry/Immunofluorescence: Calreticulin Antibody (1G6A7) [NBP1-47518] - Analysis of SKBR-3 (left) and A549 (right) cells using anti-Calreticulin mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red). DRAQ5 fluorescent DNA dye (blue).	

Flow (Intracellular): Calreticulin Antibody (1G6A7) [NBP1-47518] - A549 cells stained with NBP1-47518PCP (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to PerCP.	
Flow (Intracellular): Calreticulin Antibody (1G6A7) [NBP1-47518] - U-937 cells stained with NBP1-47518F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.	
Flow (Intracellular): Calreticulin Antibody (1G6A7) [NBP1-47518] - Jurkat cells stained with NBP1-47518APC (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to allophycocyanin.	
Flow Cytometry: Calreticulin Antibody (1G6A7) [NBP1-47518] - An intracellular stain was performed on HeLa cells with Calreticulin Antibody [1G6A7] NBP1-47518AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	

Calreticulin (1G6A7) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-Calreticulin (1G6A7) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP1-47518AF488) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Montes de Oca R, Alavi AS, Vitali N et al. Belantamab Mafodotin (GSK2857916) Drives Immunogenic Cell Death and Immune-mediated Antitumor Responses In Vivo Molecular Cancer Therapeutics 2021-10-01 [PMID: 34253590] (Flow Cytometry)

Mu L, Qi L, Long H et al. Photothermal Fibrous Chitosan/Polydopamine Sponge for Intraoperative Hemostasis and Prevention of Tumor Recurrence in Hepatocellular Carcinoma Resection Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2023-11-29 [PMID: 38029340] (FLOW)

Chang L, Chin Y, Wu P et al. Polymeric nano-formulation of spectrum selective RTK inhibitor strengthens anti-cancer effects via immune remodeling by endoplasmic reticulum stress-modulating mitochondrial metabolism Nano Today 2024-02-01 (IHC-Fr, Mouse)

Yeh YT, Sona C, Yan X et al. Restoration of PITPNA in Type 2 diabetic human islets reverses pancreatic beta-cell dysfunction Nature Communications 2023-07-17 [PMID: 37460527] (ELISA, In vivo assay)

Gu Z, Hao Y, Schomann T et al. Enhancing anti-tumor immunity through liposomal oxaliplatin and localized immunotherapy via STING activation Journal of controlled release : official journal of the Controlled Release Society 2023-04-21 [PMID: 37030544] (ICC/IF, Mouse)

Gu Z, Da Silva CG, Hao Y et al. Effective combination of liposome-targeted chemotherapy and PD-L1 blockade of murine colon cancer Journal of controlled release : official journal of the Controlled Release Society 2022-12-09 [PMID: 36460179] (ICC/IF, Mouse)

Wang H, Huang Q, Zhang Z Et al. Transient post-operative overexpression of CXCR2 on monocytes of traumatic brain injury patients drives monocyte chemotaxis toward cerebrospinal fluid and enhances monocyte-mediated immunogenic cell death of neurons in vitro J Neuroinflammation 2022-06-29 [PMID: 35768823] (FLOW, Human)

Details:
Citation using the Allophycocyanin version of this antibody.

Kim SS, Doherty C, Moghe M Et al. Nanomedicine-Based Gene Delivery for a Truncated Tumor Suppressor RB94 Promotes Lung Cancer Immunity Cancers (Basel) 2022-10-27 [PMID: 36291878] (FLOW, Human)

Details:
Citation using the Alexa Fluor 405 version of this antibody.

Kim S, Choe M, Oh J, Kim J Centrosome de-clustering of cancer cells induces cGAS-STING-mediated innate immunity of tumor-associated tumor cells in response to irradiation Biochemical and Biophysical Research Communications 2022-10-01 [PMID: 36343487] (FLOW, Human)

Kim J, Sestito L, Im S et al. Poly(cyclodextrin)-Polydrug Nanocomplexes as Synthetic Oncolytic Virus for Locoregional Melanoma Chemoimmunotherapy Adv Funct Mater 2020-10-19 [PMID: 33071710]

Li Y, Han W, He C et al. Nanoscale Coordination Polymers for Combined Chemotherapy and Photodynamic Therapy of Metastatic Cancer Bioconjug Chem 2021-10-04 [PMID: 34607430]

Details:
Citation using the Alexa Fluor 488 format of this antibody.

Bian Q, Huang L, Xu Y et al. A Facile Low-Dose Photosensitizer-Incorporated Dissolving Microneedles-Based Composite System for Eliciting Antitumor Immunity and the Abscopal Effect ACS nano 2021-12-28 [PMID: 34859990]

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Procedures

Western Blot Protocol for Calreticulin Antibody (NBP1-47518)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in 2% Non-fat milk in TBST and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three 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.

Immunocytochemistry/Immunofluorescence Protocol for Calreticulin Antibody (NBP1-47518)
Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for Calreticulin Antibody (NBP1-47518)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for Calreticulin Antibody (NBP1-47518)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NBP1-47518

NB800-PC1	HeLa Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)

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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