abcam

Product datasheet

Anti-Calreticulin antibody [EPR3924] - ER Marker ab92516

敲除验证 重组 RabMAb

6 Abreviews 76 References 18 图像

概述

产品名称	Anti-Calreticulin抗体[EPR3924] - ER Marker
描述	兔单克隆抗体[EPR3924] to Calreticulin - ER Marker
宿主	Rabbit
经测试应用	**适用于:** WB, IHC-P, Flow Cyt (Intra), ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human, African green monkey **预测可用于:** Monkey
免疫原	Synthetic peptide within Human Calreticulin aa 50-150. The exact sequence is proprietary. Database link: **P27797** (Peptide available as **ab180826**)
阳性对照	WB: SH-SY5Y, HL-60, HepG2, HeLa, Fetal kidney and Fetal brain lysates; Human kidney tissue; Mouse and Rat brain lysates. ICC/IF: HAP1 cells (HAP1-CALR as negative cell line) IHC-P: Human colon, kidney, liver, placenta, stomach, breast carcinoma and Papillary carcinoma of thyroid gland tissues; Mouse liver and Rat lung tissues.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

	supernatant
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR3924
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab92516于以下的经测试应用**

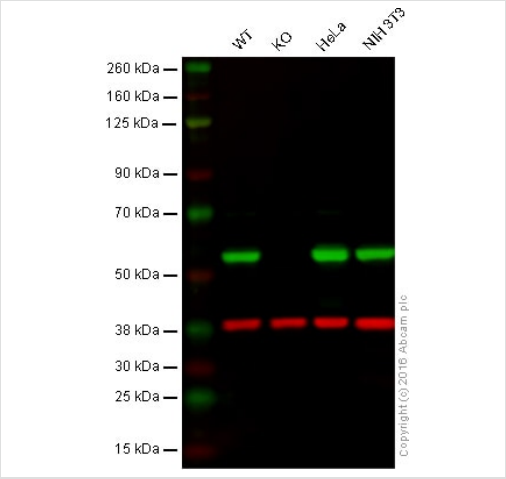
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (1)	1/1000 - 1/10000. Predicted molecular weight: 48 kDa.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The use of a HRP/AP polymerized secondary antibody is recommended for enhanced staining.
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★☆☆ (3)	1/500.

靶标

功能	Molecular calcium-binding chaperone promoting folding, oligomeric assembly and quality control in the ER via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export.
序列相似性	Belongs to the calreticulin family.
结构域	Can be divided into a N-terminal globular domain, a proline-rich P-domain forming an elongated arm-like structure and a C-terminal acidic domain. The P-domain binds one molecule of calcium with high affinity, whereas the acidic C-domain binds multiple calcium ions with low affinity. The interaction with glycans occurs through a binding site in the globular lectin domain. The zinc binding sites are localized to the N-domain. Associates with PDIA3 through the tip of the extended arm formed by the P-domain.
细胞定位	Endoplasmic reticulum lumen. Cytoplasm > cytosol. Secreted > extracellular space > extracellular matrix. Cell surface. Also found in cell surface (T cells), cytosol and extracellular matrix. Associated with the lytic granules in the cytolytic T-lymphocytes.

图片



Western blot - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

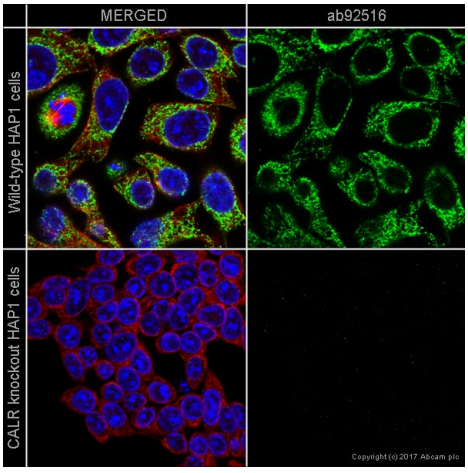
Lane 2: Calreticulin knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: NIH3T3 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab92516 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab92516 was shown to specifically react with Calreticulin when Calreticulin knockout samples were used. Wild-type and Calreticulin knockout samples were subjected to SDS-PAGE. ab92516 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

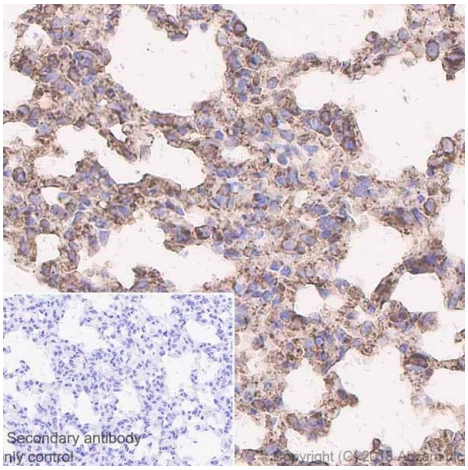


Immunocytochemistry/ Immunofluorescence - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

ab92516 staining Calreticulin in wild-type HAP1 cells (top panel) and CALR knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab92516 at 1/500 and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

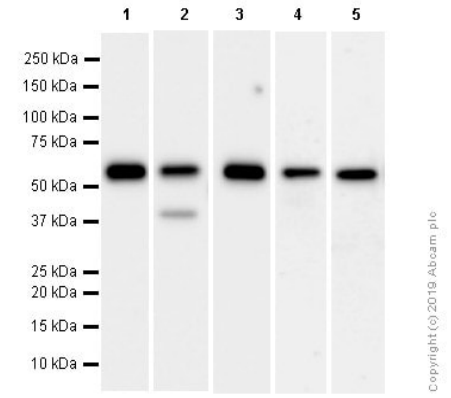
Alexa Fluor® 488 (**ab196158**) and Alexa Fluor® 647 (**ab196159**) conjugated versions are available for this clone.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on rat lung.The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Western blot - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

All lanes : Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516) at 1/10000 dilution

Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell) Whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 3 : Mouse brain lysates
Lane 4 : Rat brain lysates
Lane 5 : COS-1 (African green monkey kidney fibroblast-like) whole cell lysates

Lysates/proteins at 15 µg per lane.

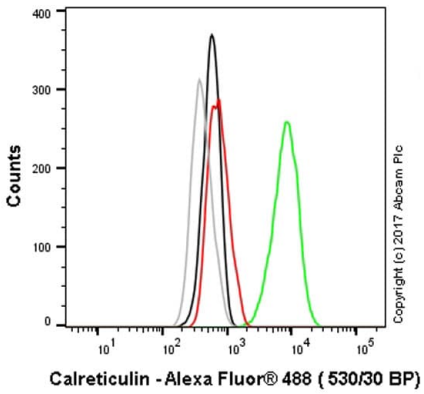
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 48 kDa

Observed band size: 55 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

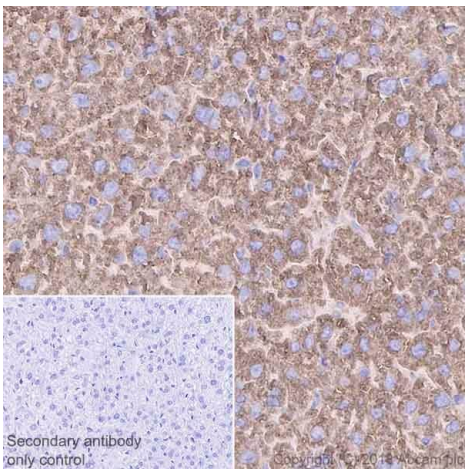
Exposure time: Lane 1 to 3: 10 seconds; Lane 4 and 5: 130 seconds



Flow Cytometry (Intracellular) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CALR knockout cells (red line) stained with ab92516. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab92516, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H&L) preadsorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CALR knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

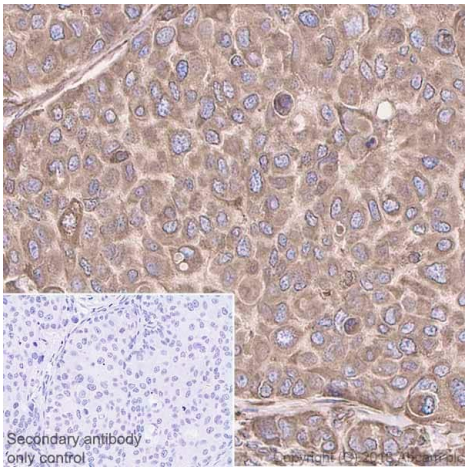
Alexa Fluorr®488 (**ab196158**) and Alexa Fluorr®647 (**ab196159**) conjugated versions are available for this clone.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on mouse liver.The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

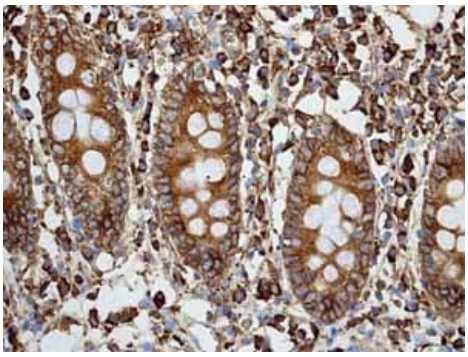
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining human breast carcinoma.The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

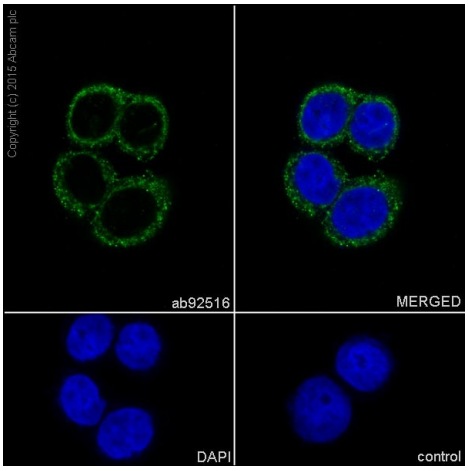
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Formalin-fixed, paraffin-embedded normal human colon tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

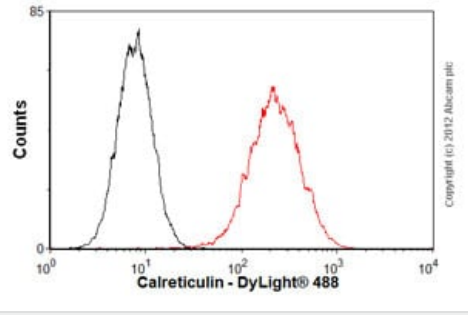
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

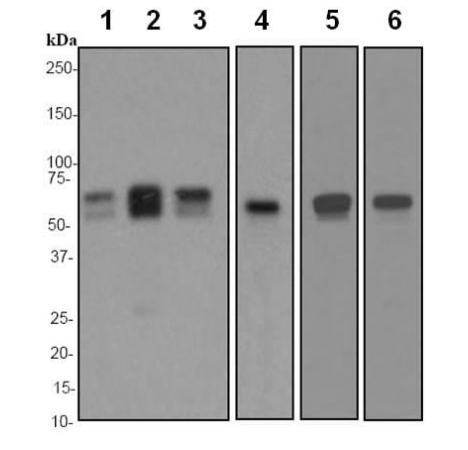
Immunocytochemistry/Immunofluorescence analysis of HT-29 (human colorectal adenocarcinoma) labelling Calreticulin with purified ab92516 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only



Flow Cytometry (Intracellular) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Overlay histogram showing HeLa cells stained with ab92516 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92516, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

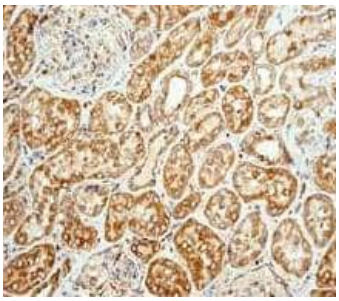
All lanes : Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516) at 1/1000 dilution

Lane 1 : SH-SY5Y cell lysate
Lane 2 : HL-60 cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : HeLa cell lysate
Lane 5 : Human fetal kidney lysate
Lane 6 : Human fetal brain lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

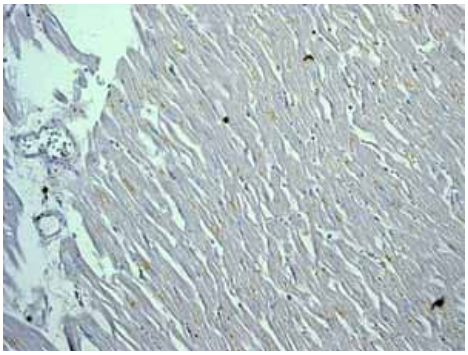
Predicted band size: 48 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

ab92516, at 1/250 dilution, staining Calreticulin in paraffin embedded Human kidney tissue by Immunohistochemistry.

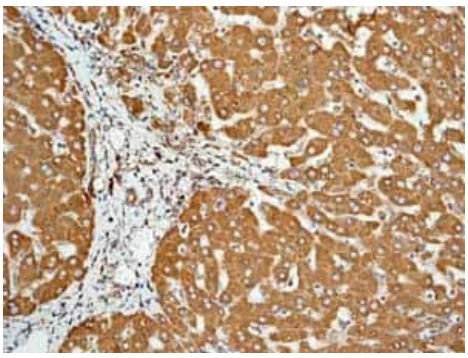
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

ab92516 showing negative staining in Normal human heart tissue.

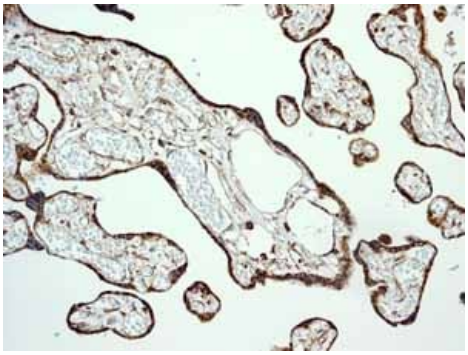
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Formalin-fixed, paraffin-embedded normal human liver tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

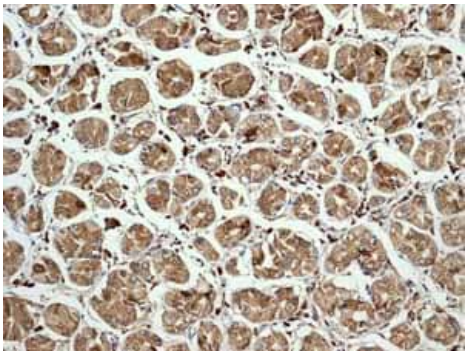
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Formalin-fixed, paraffin-embedded normal human placenta tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

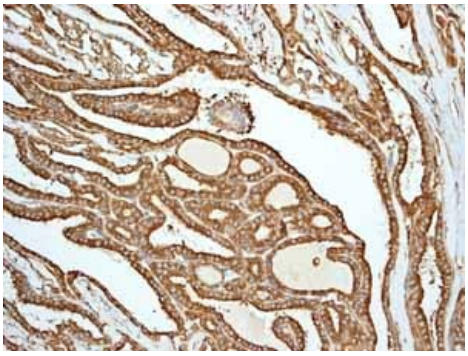
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Formalin-fixed, paraffin-embedded normal human stomach tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

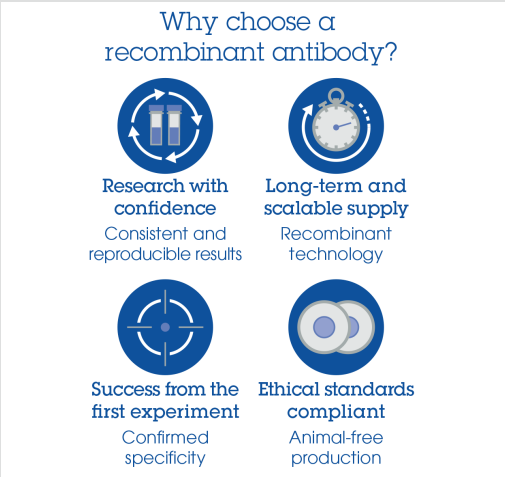
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Formalin-fixed, paraffin-embedded Papillary carcinoma of human thyroid gland tissue.stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

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