abcam

Product datasheet

Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker ab196158

KO VALIDATED Recombinant RabMAb

2 Abreviews 17 References 5 Images

Overview

Product name	Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker
Description	Alexa Fluor® 488 Rabbit monoclonal [EPR3924] to Calreticulin - ER Marker
Host species	Rabbit
Conjugation	Alexa Fluor® 488. Ex: 495nm, Em: 519nm
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab180826**)
Positive control	ICC/IF: HeLa cells, HAP1 cells (HAP1-CALR knockout cells used as negative cell line) Flow Cyt (intra): HeLa cells, HAP1-WT cells.
General notes	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**. Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research.

For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or **outlicensing@thermofisher.com**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3924
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab196158 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

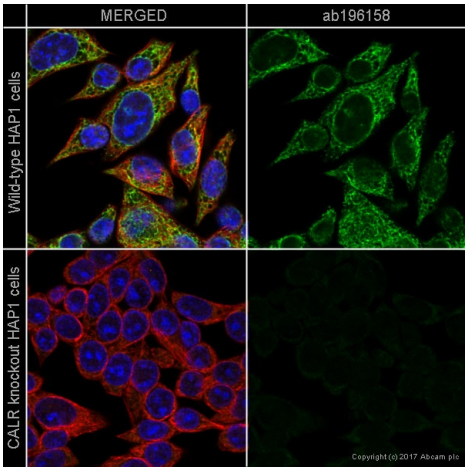
Application	Abreviews	Notes
ICC/IF		1/1000. This product gave a positive signal in HeLa cells fixed with 100% methanol (5 min)
Flow Cyt (Intra)		1/50. **ab199091** - Rabbit monoclonal IgG (Alexa FluorÂ® 488), is suitable for use as an isotype control with this antibody.

Target

Function	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.
Sequence similarities	Belongs to the calreticulin family.
Domain	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.

Cellular localization	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.

Images

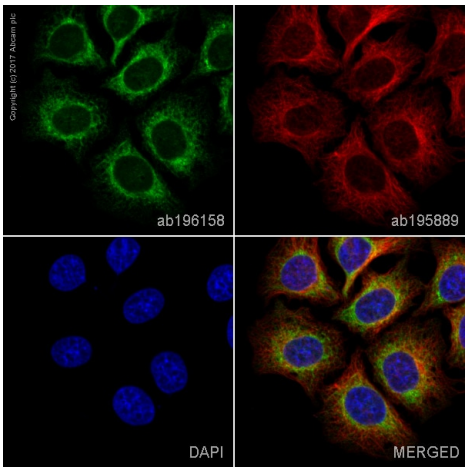


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker (ab196158)

ab196158 staining Calreticulin (shown in green) in wild-type HAP1 cells (top panel) and CALR knockout HAP1 cells (bottom panel).

The cells were fixed with 100% methanol (5 minutes), 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 1 hour. The cells were then incubated with ab196158 at 1/500 dilution (shown in green) and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C. Nuclear DNA was labeled in blue with DAPI.

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

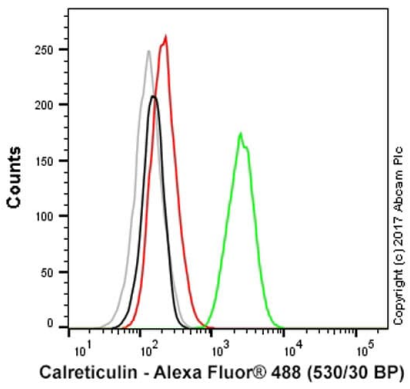


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker (ab196158)

ab196158 staining Calreticulin in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed with 100% methanol (5 minutes), 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 1 hour. The cells were then incubated overnight at +4°C with ab196158 at 1/1000 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labeled with DAPI (shown in blue).

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



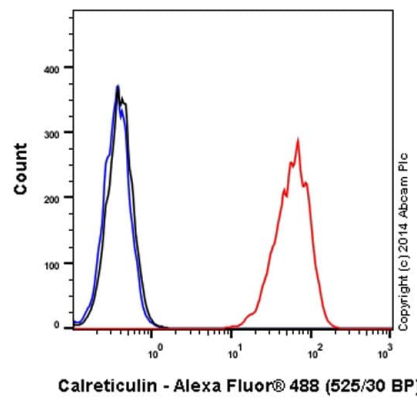
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker (ab196158)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CALR knockout cells (red line) stained with ab196158.

The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Triton X-100 for 15 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab196158, 0.1 µg/ml dilution) for 30 minutes at 22°C.

A rabbit IgG isotype control antibody (**ab199091**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CALR knockout - grey line). Unlabeled 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.

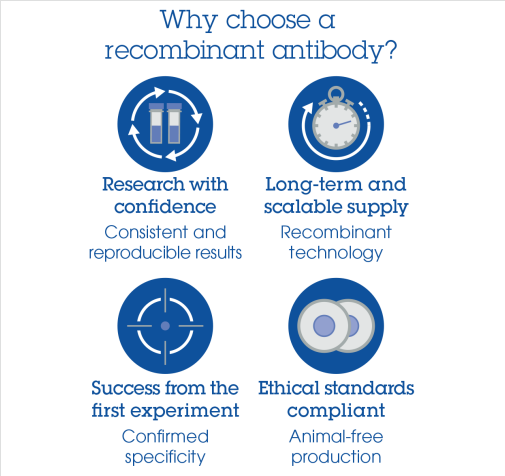


Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-Calreticulin antibody [EPR3924] - ER Marker (ab196158)

Overlay histogram showing HeLa cells (Human epithelial cell line from cervix adenocarcinoma) stained with ab196158 (red line).

The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. 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 (ab196158, 1/50 dilution) for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



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