abcam

Product datasheet

Anti-Calnexin antibody [6F12BE10] ab112995

KO VALIDATED

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Overview

Product name	Anti-Calnexin antibody [6F12BE10]
Description	Mouse monoclonal [6F12BE10] to Calnexin
Host species	Mouse
Specificity	Shotgun immunization of human HeLa cell lysates into mice. Targets were determined by mass spectrometry and validated by WB, ICC, ELISA pair and other techniques.
Tested applications	**Suitable for:** WB, IP, Flow Cyt, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Immunogen	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa cells; HL60 cells and human fibroblasts.
General notes	This monoclonal antibody to calnexin has been knockout validated in WB and ICC/IF. The expected signal was observed in wild type cells and the signal was not seen in knockout cells. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.
Storage buffer	pH: 7.5 Preservative: 0.02% Sodium azide Constituent: 99.98% HEPES buffered saline

Purity	Proprietary Purification
Purification notes	ab112995 was produced in vitro using hybridomas grown in serum-free medium, and then purified by biochemical fractionation. Monoclonal purity was near homogeneity as judged by SDS-PAGE (>95%).
Clonality	Monoclonal
Clone number	6F12BE10
Isotype	IgG2b
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab112995 in the following tested applications.

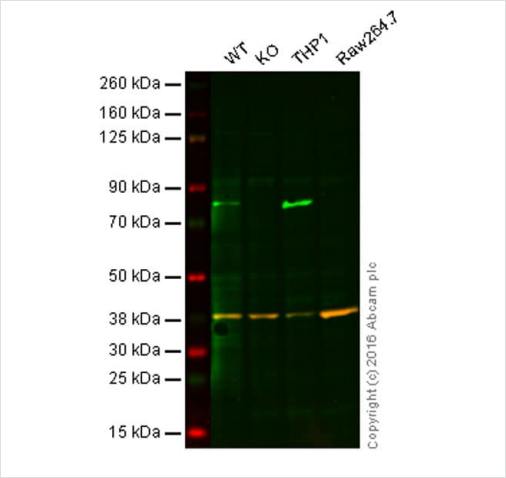
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★☆☆ (1)	Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.
IP		Use at an assay dependent concentration.
Flow Cyt		Use a concentration of 1 µg/ml. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
ICC/IF		Use a concentration of 0.5 µg/ml.
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function	Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins.
Sequence similarities	Belongs to the calreticulin family.
Cellular localization	Endoplasmic reticulum membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

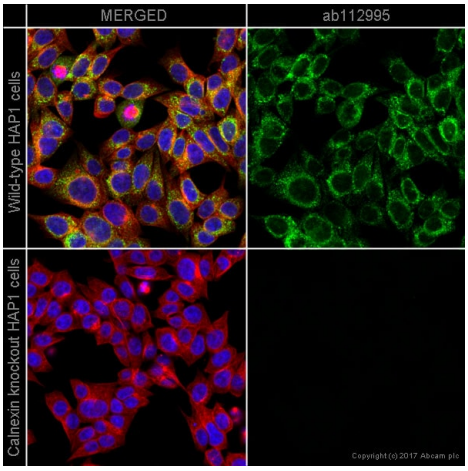
Images



Western blot - Anti-Calnexin antibody [6F12BE10] (ab112995)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Calnexin knockout HAP1 cell lysate (20 µg)
Lane 3: THP1 cell lysate (20 µg)
Lane 4: Raw264.7 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab112995 observed at 80 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

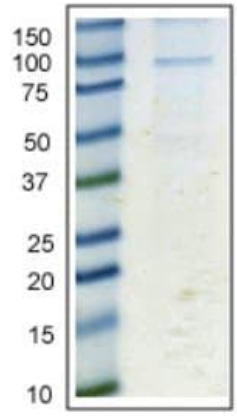
ab112995 was shown to specifically react with Calnexin. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. ab112995 at a concentration of 1 µg/mL and **ab181602** (loading control to GAPDH) diluted to 1/1000 were incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody [6F12BE10] (ab112995)

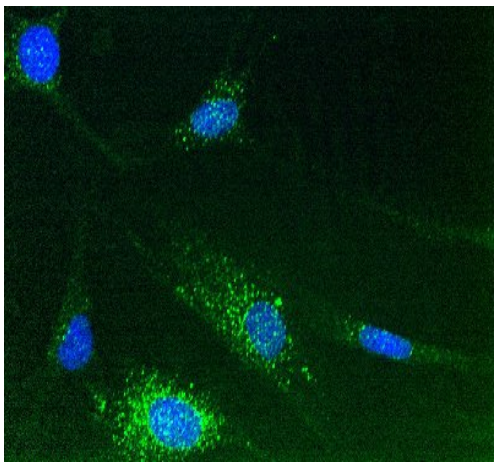
ab112995 staining Calnexin (shown in green) in wild-type HAP1 cells (top panel) and CANX knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 min and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated with ab112995 at 0.5 µg/ml and **ab202272** at 1/250 dilution (alpha tubulin shown in red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat secondary antibody to Mouse IgG (Alexa Fluor® 488) (**ab150117**) 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).



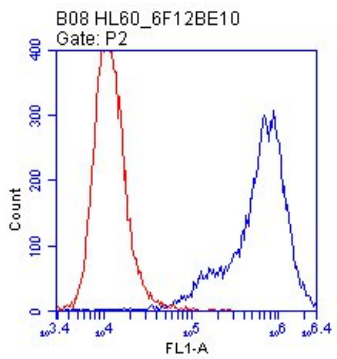
Immunoprecipitation - Anti-Calnexin antibody [6F12BE10] (ab112995)

Immunoprecipitation with ab112995.
Immunoprecipitation of Calnexin - ER membrane marker from HeLa cell lysate. The protein band runs around 90 kDa (predicted 68kDa) in tris-glycine SDS-PAGE. The identity of this protein was confirmed by mass spectrometry. This gel was stained with colloidal Coomassie blue G.



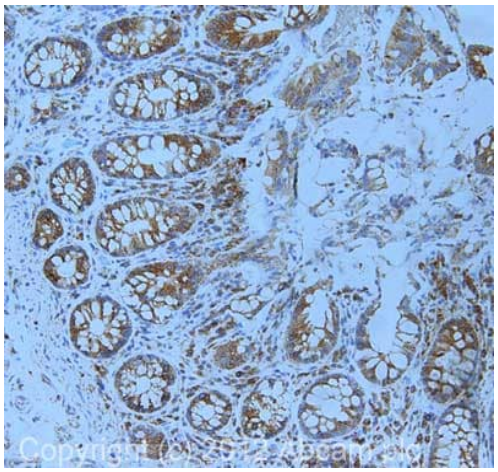
Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody [6F12BE10] (ab112995)

ab112995 at 5µg/ml staining Calnexin - ER membrane marker in Human fibroblasts cells by Immunocytochemistry (4% paraformaldehyde fixed and 0.1% Triton X-100 permeabilized) followed by Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h (green).



Flow Cytometry - Anti-Calnexin antibody [6F12BE10] (ab112995)

ab112995 at 1µg/ml staining Calnexin - ER membrane marker in HL60 cells fixed with MeOH by Flow Cytometry (blue). Isotype control antibody (red).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [6F12BE10] (ab112995)

IHC image of ab112995 staining in human colon formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab112995, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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