abcam

Product datasheet

Anti-KDEL antibody [EPR12668] ab176333

Recombinant RabMAb

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Overview

Product name	Anti-KDEL antibody [EPR12668]
Description	Rabbit monoclonal [EPR12668] to KDEL
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	293T, MCF-7, Raji, HepG2, HeLa, Jurkat, and K562 cell lysates. MCF7 and HeLa cells. Paraffin-embedded Human heart and kidney tissue.
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**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.06% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR12668
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab176333 in the following tested applications.

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

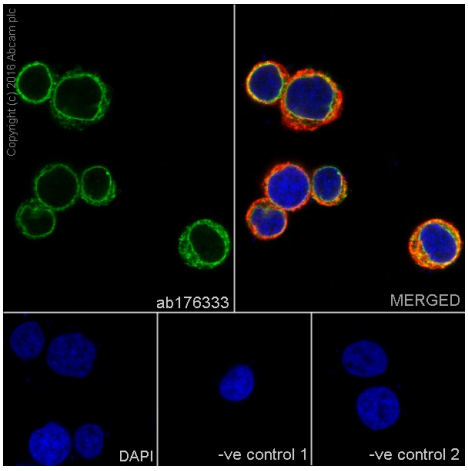
Application	Abreviews	Notes
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (2)	1/1000 - 1/10000. Predicted molecular weight: 25 kDa.
IHC-P		1/50 - 1/350. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ (1)	1/100 - 1/250.

Target

Relevance The sequence Lys-Asp-Glu-Leu (KDEL) or a closely related sequence, is present at the carboxy-terminus of soluble endoplasmic reticulum (ER) resident proteins and some membrane proteins. 78 and 94 kDa glucose regulated proteins (GRP 78) and GRP 94 respectively and protein disulfide isomerase (PDI) all share the C-terminal KDEL sequence. The presence of carboxy-terminal KDEL appears to be necessary for ER retention and appears to be sufficient to reduce the secretion of proteins from the ER. This retention is reported to be mediated by a KDEL receptor.

Cellular localization Endoplasmic reticulum

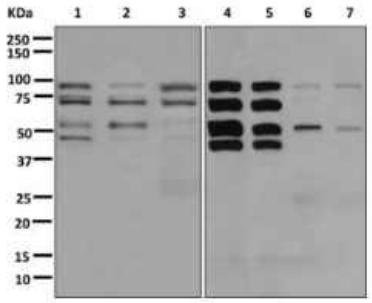
Images



Immunocytochemistry/ Immunofluorescence - Anti-KDEL antibody [EPR12668] (ab176333)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling KDEL with purified ab176333 at 1/200. Cells were fixed with 4% Paraformaldehyde and permeabilized using 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were co-stained with **ab7291**, a mouse anti-tubulin antibody (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary. Nuclei were counterstained with DAPI (blue).

For negative control 1, **ab150120** (Rabbit primary antibody) and anti-mouse secondary antibody were used and for negative control 2, **ab7291** (Mouse primary antibody) and **ab150077** (anti-rabbit secondary antibody) were used.



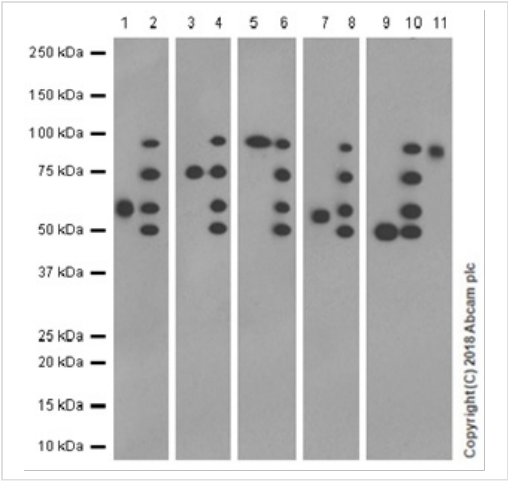
Western blot - Anti-KDEL antibody [EPR12668] (ab176333)

All lanes : Anti-KDEL antibody [EPR12668] (ab176333) at 1/1000 dilution

Lane 1 : 293T cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : HepG2 cell lysate
Lane 5 : HeLa cell lysate
Lane 6 : Jurkat cell lysate
Lane 7 : K562 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 25 kDa



Western blot - Anti-KDEL antibody [EPR12668] (ab176333)

Lane 1 : Anti-P4HB antibody [EPR9498] (**ab137119**) at 1/5000 dilution
Lanes 2 & 4 & 6 & 8 & 10 : Anti-KDEL antibody [EPR12668] (ab176333) at 1/5000 dilution
Lane 3 : Anti-GRP78 BiP antibody [EPR4040(2)] (**ab108613**) at 1/5000 dilution
Lane 5 : Anti-GRP94 antibody [EPR3988] (**ab108606**) at 1/5000 dilution
Lane 7 : Anti-ERp57 antibody [EPR10678(B)] (**ab154191**) at 1/5000 dilution
Lane 9 : Anti-PDIA6 antibody [EPR10132(B)] (**ab154820**) at 1/5000 dilution
Lane 11 : Anti-LEPRE1/P3H1 antibody [EPR10193(B)] (**ab154799**) at 1/5000 dilution

All lanes : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate with 5% NFDM/TBST

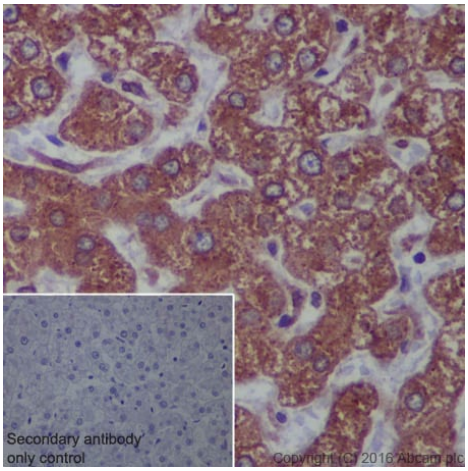
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Predicted band size: 25 kDa

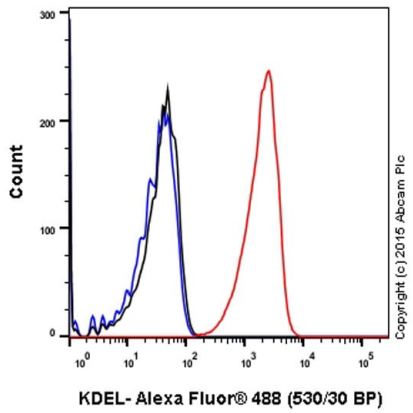
Exposure time: 3 minutes

ab176333 detected several bands which have similar MWs with some proteins containing carboxyl terminal KDEL motifs, including P4HB, GRP78 BiP, GRP94, PDIA6 and LEPRE1 (PMID: 25683117, PMID: 19741001, PMID: 22079671, PMID: 28648146, PMID: 22615817). It indicates that ab176333 might recognize these proteins.



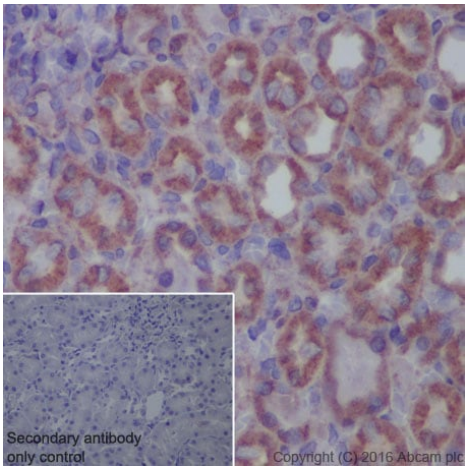
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDEL antibody [EPR12668] (ab176333)

Immunohistochemical analysis of paraffin-embedded human liver sections labelling KDEL with purified ab176333 at dilution of 1:350. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



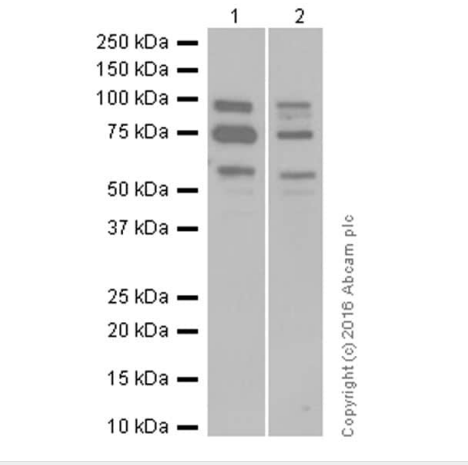
Flow Cytometry (Intracellular) - Anti-KDEL antibody [EPR12668] (ab176333)

Overlay histogram showing 4% paraformaldehyde fixed HeLa cells labelling KDEL (red) with purified ab176333 at dilution of 1/100. The secondary antibody used was Alexa Fluor® 488 goat-anti-rabbit IgG at dilution of 1/2000. A non-specific IgG antibody (rabbit monoclonal) was used as isotype control (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDEL antibody [EPR12668] (ab176333)

Immunohistochemical analysis of paraffin-embedded rat kidney sections labelling KDEL with purified ab176333 at dilution of 1:350. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Western blot - Anti-KDEL antibody [EPR12668] (ab176333)

All lanes : Anti-KDEL antibody [EPR12668] (ab176333) at 1/10000 dilution

Lane 1 : Raw264.7 whole cell lysate
Lane 2 : C6 whole cell lysate

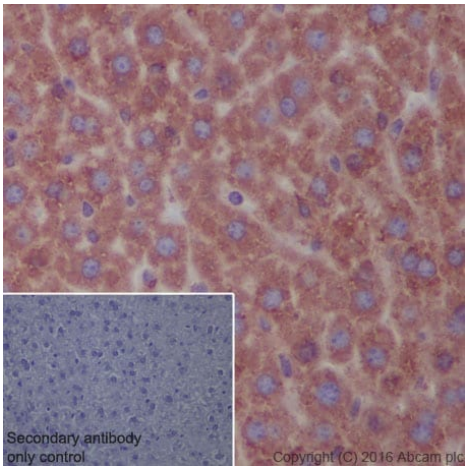
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 25 kDa

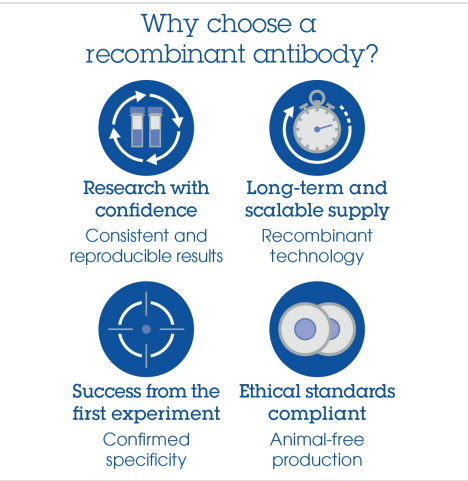
Observed band size : 94,78,57,48

Blocking/Diluting buffer 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDEL antibody [EPR12668] (ab176333)

Immunohistochemical analysis of paraffin-embedded mouse liver sections labelling KDEL with purified ab176333 at dilution of 1:350. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Anti-KDEL antibody [EPR12668] (ab176333)

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