abcam

Product datasheet

Anti-GRP78 BiP antibody ab21685

71 Abreviews 553 References 9 Images

Overview

Product name	Anti-GRP78 BiP antibody
Description	Rabbit polyclonal to GRP78 BiP
Host species	Rabbit
Specificity	Replenishment batches of our polyclonal antibody, ab21685 are tested in WB. Previous batches were additionally validated in ICC/IF and IHC-P. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab108613**.
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human, Chinese hamster **Predicted to work with:** Dog, Pig, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	Stimulation may be required to allow detection of the target protein due to low levels of endogenous expression in some samples. Please see images below for recommended treatment conditions and positive controls. 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). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab21685 in the following tested applications.

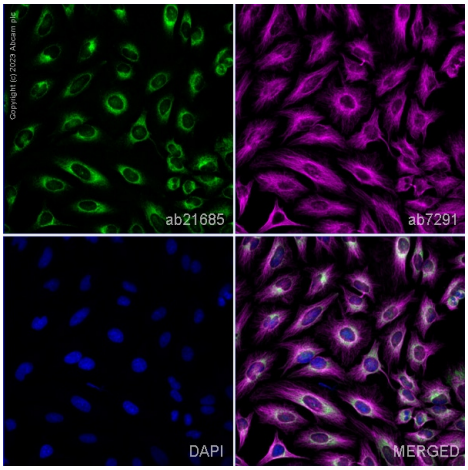
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ **(25)**	Use a concentration of 1 - 5 µg/ml. We recommend using **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody**.
WB	★★★★★ **(31)**	Use a concentration of 1 µg/ml. Detects a band of approximately 75 kDa (predicted molecular weight: 78 kDa).
IHC-P	★★★★★ **(4)**	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the endoplasmic reticulum. Involved in the correct folding of proteins and degradation of misfolded proteins via its interaction with DNAJC10, probably to facilitate the release of DNAJC10 from its substrate.
Involvement in disease	Autoantigen in rheumatoid arthritis.
Sequence similarities	Belongs to the heat shock protein 70 family.
Cellular localization	Endoplasmic reticulum lumen. Melanosome. Cytoplasm. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images

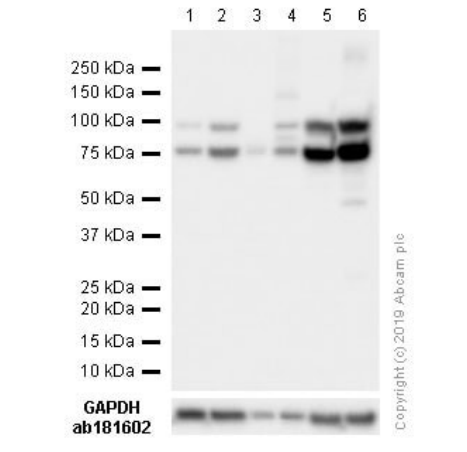


Immunocytochemistry/ Immunofluorescence - Anti-GRP78 BiP antibody (ab21685)

ab21685 staining GRP78 BiP in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab21685 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-GRP78 BiP antibody (ab21685)

All lanes : Anti-GRP78 BiP antibody (ab21685) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa treated with 2.5 µg/ml tunicamycin for 24h whole cell lysates
Lane 3 : HUVEC (Human umbilical vein endothelial cell) whole cell lysates
Lane 4 : HUEVC (Human umbilical vein endothelial cell) treated with 10 µg/ml tunicamycin for 48h whole cell lysates
Lane 5 : Raw 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 6 : Raw 264.7 treated with 5 µg/ml tunicamycin for 18h whole cell lysates

Lysates/proteins at 20 µg per lane.

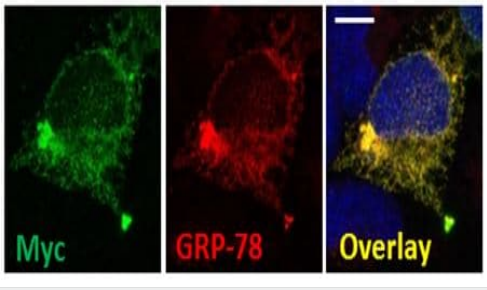
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at

1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 78 kDa
Observed band size: 78 kDa

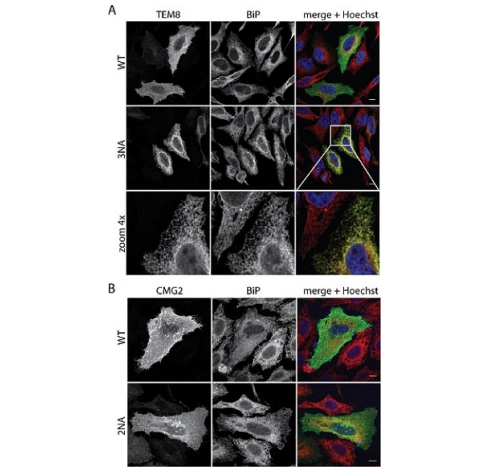
Exposure time: 3 seconds

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



Immunocytochemistry/ Immunofluorescence - Anti-GRP78 BiP antibody (ab21685)

HEK-293 cells were cultured on coverslips and transiently transfected with wild-type murine IGSF1-2-Myc/His, premeabilized, and processed for double-label immunofluorescence with the Myc antibody (green) and an antibody against GRP78 BiP (red). The overlay is shown in yellow. Nuclei were stained with DAPI. Images were captured by confocal microscopy. Scale bar, 10 µm.

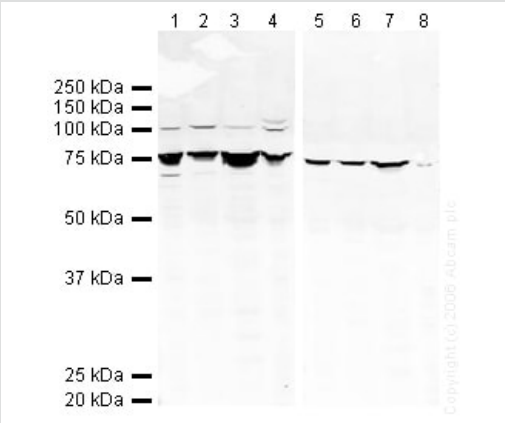


Immunocytochemistry/ Immunofluorescence - Anti-GRP78 BiP antibody (ab21685)

Localization of TEM8 and CMG2 glycosylation mutants.

A) Immunofluorescence of transiently transfected HeLa cells. Cells were transfected for 48h with the respective cDNAs. Cells were fixed, permeabilized and stained for TEM8-HA, endogenous BiP and Hoechst. Scalebars represent 10 µm.

B) Immunofluorescence of transiently transfected HeLa cells. Cells were transfected for 48h with the respective cDNAs. Cells were fixed, permeabilized and stained for CMG2-V5, endogenous BiP and Hoechst. Scalebars represent 10 µm.



Western blot - Anti-GRP78 BiP antibody (ab21685)

All lanes : Anti-GRP78 BiP antibody (ab21685) at 1 µg/ml

Lane 1 : CHO-K1 (chinese hamster ovary cell line) whole cell lysate at 20 µg
Lane 2 : Liver (Mouse) Tissue Lysate at 20 µg
Lane 3 : Rat liver whole cell lysate at 20 µg
Lane 4 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg
Lane 5 : CHO-K1 whole cell lysate at 20 µg/ml with Mouse GRP78 BiP peptide (**ab22410**) at 1 µg
Lane 6 : Liver (Mouse) Tissue Lysate at 20 µg with Mouse GRP78 BiP peptide (**ab22410**) at 1 µg/ml
Lane 7 : Rat liver whole cell lysate at 20 µg with Mouse GRP78 BiP peptide (**ab22410**) at 1 µg/ml
Lane 8 : HeLa whole cell lysate at 20 µg with Mouse GRP78 BiP peptide (**ab22410**) at 1 µg/ml

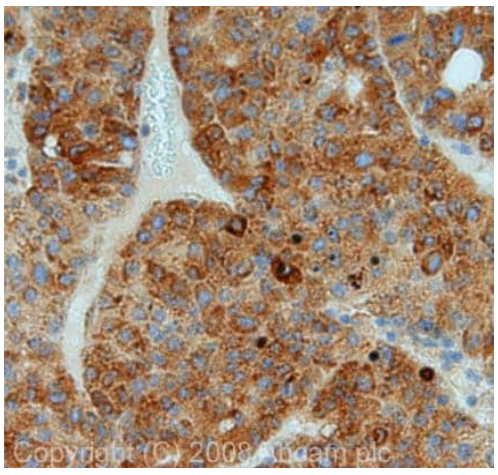
Secondary
All lanes : Goat anti Rabbit IgG at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 78 kDa
Observed band size: 75 kDa
Additional bands at: 100 kDa. We are unsure as to the identity of these extra bands.

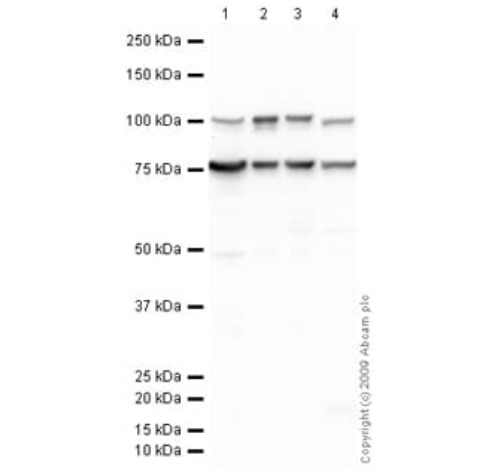
ab21685 recognises a band of ~ 75 kDa in CHO, mouse liver, rat liver and HeLa whole cell lysates, corresponding to GRP78 BiP. This band is quenched by the addition of the immunizing peptide, **ab22410**.

ab21685 also detects a 100 kDa band in Western Blot. We are unsure of the identity of this protein.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GRP78 BiP antibody (ab21685)

IHC image of GRP78 BiP staining in human liver carcinoma FFPE section, performed on a Bond^{TM} 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 ab21685, 1µg/ml, for 8 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.



Western blot - Anti-GRP78 BiP antibody (ab21685)

All lanes : Anti-GRP78 BiP antibody (ab21685) at 1 µg/ml

Lane 1 : CHO-K1 (chinese hamster ovary cell line) Whole Cell Lysate
Lane 2 : Liver (Mouse) Tissue Lysate at 10 µg
Lane 3 : Liver (Rat) Tissue Lysate at 10 µg
Lane 4 : HeLa (human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Secondary
Lanes 1 & 4 : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution
Lanes 2-3 : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 78 kDa
Observed band size: 78 kDa
Additional bands at: 100 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 1 minute

Western blot - Anti-GRP78 BiP antibody (ab21685)

All lanes : Anti-GRP78 BiP antibody (ab21685) at 1 µg/ml

Lane 1 : Recombinant human GRP78 BiP protein (Active) (**ab78432**) at 0.1 µg
Lane 2 : Recombinant human GRP78 BiP protein (Active) (**ab78432**) at 0.01 µg

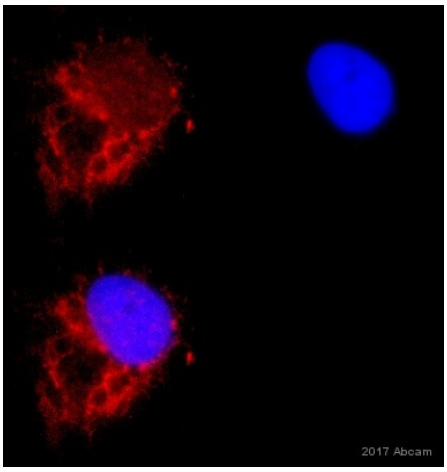
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 78 kDa

Exposure time: 10 seconds



Immunocytochemistry/ Immunofluorescence - Anti-GRP78 BiP antibody (ab21685)
This image is courtesy of an anonymous abreview.

Immunocytochemistry/ Immunofluorescence analysis of MDA-MB-435S tumor cell line cells labeling GRP78 BiP with ab21685 at 1/400 dilution. Cells were fixed in formaldehyde and permeabilized with 0.25% Triton-X100 in PBS for 10 minutes. Blocking was done with 1%BSA for 1 hour at 20°C; followed by staining with ab21685 at 1/400 for 18 hours. Undiluted **ab150077**, a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) secondary antibody was used. DAPI was used to counterstain.

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