abcam

Product datasheet

Anti-Cyclophilin B antibody ab16045

KO VALIDATED

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Overview

Product name	Anti-Cyclophilin B antibody
Description	Rabbit polyclonal to Cyclophilin B
Host species	Rabbit
Specificity	Replenishment batches of our polyclonal antibody, ab16045 are tested in WB. Previous batches were additionally validated in ICC/IF and IP. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab178397**.
Tested applications	**Suitable for:** WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Horse, Chicken, Dog, Human **Predicted to work with:** Cow, Pig, Xenopus laevis
Immunogen	Synthetic peptide corresponding to Human Cyclophilin B aa 150 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab16277**, **ab5016**)
General notes	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 ab16045 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	★★★★★ (7)	Use a concentration of 0.5 µg/ml. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
ICC/IF	★★★★★ (3)	Use a concentration of 1 µg/ml.
IP		Use at an assay dependent concentration.

Target

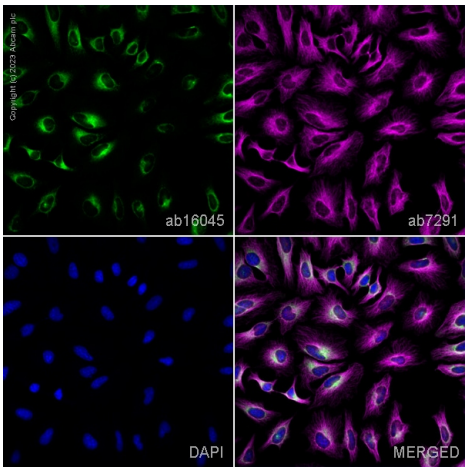
Function PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides.

Involvement in disease Defects in PPIB are the cause of osteogenesis imperfecta type 9 (OI9) [MIM:259440]. OI9 is a connective tissue disorder characterized by bone fragility, low bone mass and bowing of limbs due to multiple fractures. Short limb dwarfism and blue sclerae are observed in some but not all patients.

Sequence similarities Belongs to the cyclophilin-type PPIase family. PPIase B subfamily.
Contains 1 PPIase cyclophilin-type domain.

Cellular localization Endoplasmic reticulum lumen. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

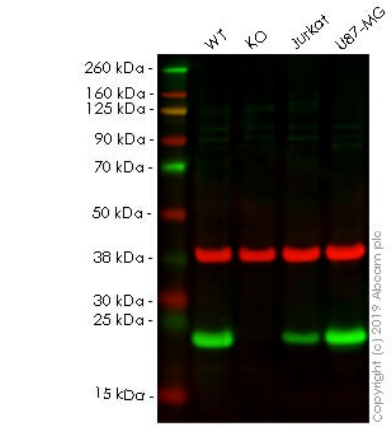
Images



Immunocytochemistry/ Immunofluorescence - Anti-Cyclophilin B antibody (ab16045)

ab16045 staining Cyclophilin B 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 ab16045 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).

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-Cyclophilin B antibody (ab16045)

All lanes : Anti-Cyclophilin B antibody (ab16045) at 1/5000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : PPIB knockout HAP1 whole cell lysate
Lane 3 : Jurkat whole cell lysate
Lane 4 : U87-MG whole cell lysate

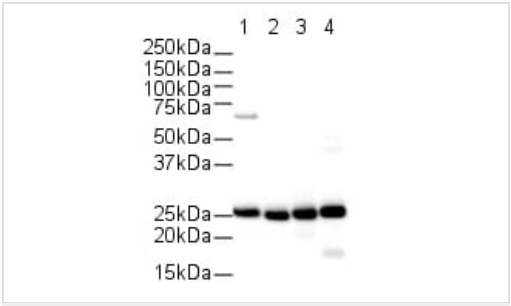
Lysates/proteins at 20 µg per lane.

Predicted band size: 21 kDa
Observed band size: 24 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab16045 observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab16045 was shown to specifically react with PPIB in wild-type HAP1 cells as signal was lost in PPIB knockout cells. Wild-type and

PPIB knockout samples were subjected to SDS-PAGE. Ab16045 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Cyclophilin B antibody (ab16045)

All lanes : Anti-Cyclophilin B antibody (ab16045) at 1 µg/ml

Lane 1 : Rat Liver
Lane 2 : Mouse 3T3
Lane 3 : Dog
Lane 4 : Chicken

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 25 kDa

Exposure time: 30 seconds

Western blot - Anti-Cyclophilin B antibody (ab16045)

All lanes : Anti-Cyclophilin B antibody (ab16045) at 1 µg/ml

Lane 1 : HeLa nuclear lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : A431 whole cell lysate
Lane 4 : Jurkat whole cell lysate
Lane 5 : HEK293 whole cell lysate
Lane 6 : HeLa nuclear lysate with Human Cyclophilin B peptide (**ab16277**) at 1 µg/ml
Lane 7 : HeLa whole cell lysate with Human Cyclophilin B peptide (**ab16277**) at 1 µg/ml
Lane 8 : A431 whole cell lysate with Human Cyclophilin B peptide (**ab16277**) at 1 µg/ml
Lane 9 : Jurkat whole cell lysate with Human Cyclophilin B peptide

(**ab16277**) at 1 µg
Lane 10 : HEK293 whole cell lysate with Human Cyclophilin B peptide (**ab16277**) at 1 µg/ml

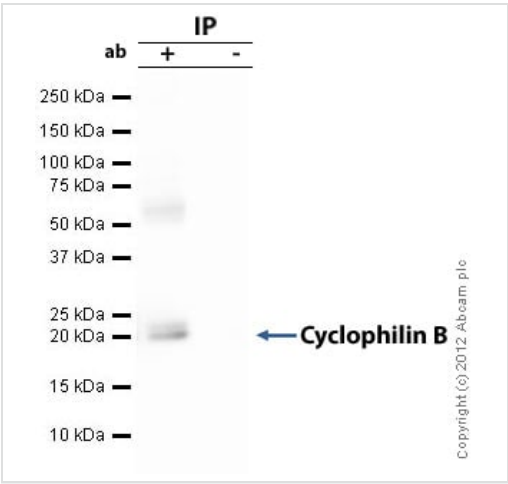
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Performed under reducing conditions.

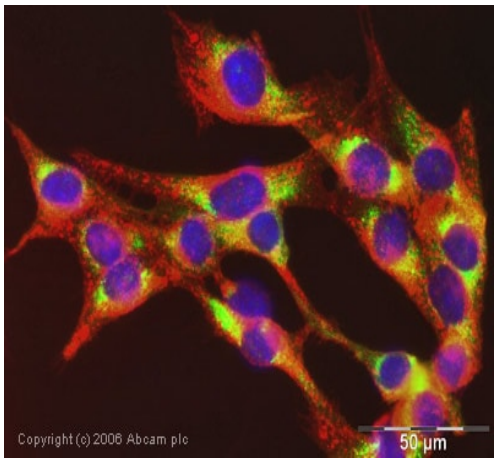
Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 30 seconds



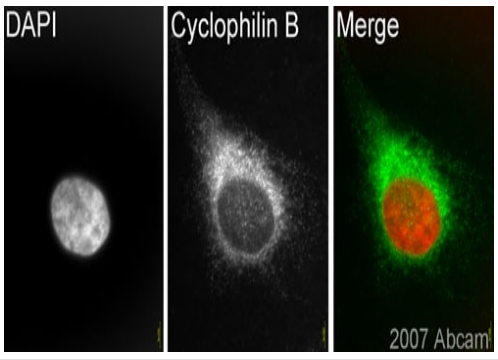
Immunoprecipitation - Anti-Cyclophilin B antibody (ab16045)

Cyclophilin B was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Cyclophilin B and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab16045.
Secondary: Clean blot (HRP conjugate) at 1/1000 dilution.
Band: 21kDa: Cyclophilin B.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclophilin B antibody (ab16045)

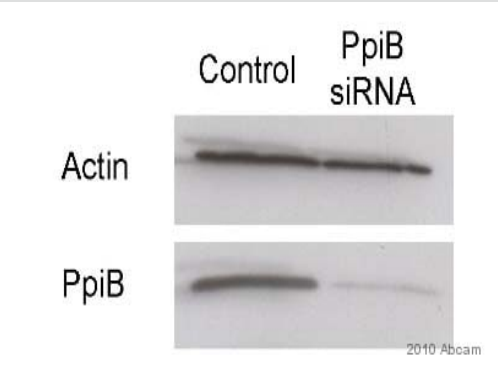
ICC/IF image of ab16045 stained NIH/3T3 cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab16045, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Immunocytochemistry/ Immunofluorescence - Anti-Cyclophilin B antibody (ab16045)

This image is courtesy of an Abreview submitted by Dr Kirk McManus

ab16045 (1/1000) staining Cyclophilin B in assynchronous HeLa cells (green). Cells were fixed with Paraformaldehyde and counter-stained with DAPI in order to highlight the nucleus (red). Please refer to abreview for further experimental details.



Western blot - Anti-Cyclophilin B antibody (ab16045)

Image courtesy of Dr M Schrader by Abreview.

All lanes : Anti-Cyclophilin B antibody (ab16045) at 1/1000 dilution

Lane 1 : Whole cell lysate prepared from rat pancreatic AR42J cells, which were treated with 10nM dexamethasone for 48 hours.
Lane 2 : Whole cell lysate for negative control, prepared from rat pancreatic AR42J cells (specific knock down of cyclophilin B/PpiB by siRNA), which were treated with 10nM dexamethasone for 48 hours.

Secondary
All lanes : Goat-anti-Rabbit HRP-conjugated polyclonal at 1/2000 dilution

Developed using the ECL technique.

Predicted band size: 21 kDa

Observed band size: 23 kDa

Exposure time: 1 minute

Primary antibody incubated for 12 hours at 4°C.
Blocking step performed using 5% milk, 1 hour at 20°C.

Western blot - Anti-Cyclophilin B antibody (ab16045)

Anti-Cyclophilin B antibody (ab16045) at 0.5 µg/ml + Recombinant Human Cyclophilin B protein (**ab88801**) at 0.01 µg

Secondary
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: 21 kDa

Exposure time: 30 seconds

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