Product datasheet

Anti-Cyclophilin B antibody ab16045

Overview

Product name: Anti-Cyclophilin B antibody
Description: Rabbit polyclonal to Cyclophilin B
Host species: Rabbit
Tested applications: Suitable for: WB, IHC-P, ICC/IF, IHC-Fr, 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)

Positive control: WB: HeLa, HeLa Nuclear, Jurkat, A431, HEK293, NIH 3T3, HAP1 and U87-MG cell lysates; Rat Liver Tissue IF: NIH3T3 cells

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

Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

12 Abreviews  60 References  10 Images
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

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

why is the actual band size different from the predicted?

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.5 µg/ml. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa). Can be blocked with Human Cyclophilin B peptide (ab16277).</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>
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.

Immunocytochemistry/ Immunofluorescence - Anti-Cyclophilin B antibody (ab16045) stained HeLa cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab16045 at 5µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) used at a 1/1000 dilution for 1 hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1 hour at room temperature.

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  
*why is the actual band size different from the predicted?*

**Exposure time:** 30 seconds

**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
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°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.

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).

ab16045 (1/1000) staining Cyclophilin B in asynchronous 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.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclophilin B antibody (ab16045)

Image courtesy of Human Protein Atlas

ab16045 staining in human placenta, showing staining of the cytotrophoblasts. Paraffin embedded placental tissue was incubated with ab16045 (1:14,000 dilution) for 30 mins at room temperature. Antigen retrieval was performed by heat induction in citrate buffer pH 6. ab16045 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines. Further results for this antibody can be found at www.proteinatlas.org.

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

why is the actual band size different from the predicted?

Exposure time: 1 minute

Primary antibody incubated for 12 hours at 4°C.

Blocking step performed using 5% milk, 1 hour at 20°C.
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

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors