Product datasheet

Anti-Bcl-XL antibody [E18] ab32370

Product name: Anti-Bcl-XL antibody [E18]
Description: Rabbit monoclonal [E18] to Bcl-XL
Host species: Rabbit
Specificity: This antibody should recognize Bcl-XL, Bcl-xS and Bcl-x(beta) as the immunogen sequence is common to them. The antibody does not cross-react with other Bcl-2 family members.

Tested applications: Suitable for: WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Pig

Immunogen: Synthetic peptide within Human Bcl-XL aa 1-100. The exact sequence is proprietary.
Database link: Q07817


General notes: A trial size is available to purchase for this antibody.
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid
Dissociation constant (K_D): K_D = 6.50 x 10^{-11} M
**Storage buffer**  
- pH: 7.20  
- Preservative: 0.01% Sodium azide  
- Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

**Purity**  
- Protein A purified

**Clonality**  
- Monoclonal

**Clone number**  
- E18

**Isotype**  
- IgG

**Applications**

Our Abpromise guarantee covers the use of ab32370 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>
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<tbody>
<tr>
<td>WB</td>
<td>★★★☆☆☆ercially available online</td>
<td>1/1000. Detects a band of approximately 26 kDa.</td>
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<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100 - 1/500.</td>
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<tr>
<td>Flow Cyt</td>
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<td>1/20 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/10 - 1/30.</td>
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**Target**

**Function**  
- Potent inhibitor of cell death. Inhibits activation of caspases (By similarity). Appears to regulate cell death by blocking the voltage-dependent anion channel (VDAC) by binding to it and preventing the release of the caspase activator, CYC1, from the mitochondrial membrane.
- Isoform Bcl-X(S) promotes apoptosis.

**Tissue specificity**  
- Bcl-X(S) is expressed at high levels in cells that undergo a high rate of turnover, such as developing lymphocytes. In contrast, Bcl-X(L) is found in tissues containing long-lived postmitotic cells, such as adult brain.

**Sequence similarities**  
- Belongs to the Bcl-2 family.

**Domain**  
- The BH4 motif is required for anti-apoptotic activity. The BH1 and BH2 motifs are required for both heterodimerization with other Bcl-2 family members and for repression of cell death.

**Post-translational**  
- Proteolytically cleaved by caspases during apoptosis. The cleaved protein, lacking the BH4 motif,
modifications
has pro-apoptotic activity.

Cellular localization

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium tissue labelling Bcl-XL with purified ab32370 at 1/2000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit HRP (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunocytochemistry/Immunofluorescence analysis of C6(Rat glial tumor glial cell) cells labeling Bcl-XL with purified ab32370 at 1/100 dilution (1.42 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 dilution (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
Immunocytochemistry/ Immunofluorescence analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling Bcl-XL with purified ab32370 at 1/100 dilution (1.42 μg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 dilution (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunoprecipitation - Anti-Bcl-XL antibody [E18] (ab32370)

ab32370 (purified) at 1/500 dilution (0.28 μg/ml) immunoprecipitating Bcl-XL in NIH/3T3 whole cell lysate.

Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10µg
Lane 2 (+): ab32370 & NIH/3T3 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32370 in NIH/3T3 whole cell lysate

For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used as the secondary antibody at 1/1000 dilution. Blocking and diluting buffer: 5% NFDM /TBST.
Flow cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labelling Bcl-XL with ab32370 at 1/20 dilution (7.1 µg/ml) (Red). Cells were fixed with 4% paraformaldehyde. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlabeled control - Unlabelled cells (Blue).

Flow cytometry analysis of C6 (Rat glial tumor glial cell) cells labelling Bcl-XL with ab32370 at 1/20 dilution (7.1 µg/ml) (Red). Cells were fixed with 4% paraformaldehyde. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlabeled control - Unlabelled cells (Blue).
All lanes: Anti-Bcl-XL antibody [E18] (ab32370) at 1/1000 dilution (purified)

Lane 1: Jurkat cell lysate
Lane 2: K562 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Observed band size: 26 kDa

why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

ICC/IF image of unpurified ab32370 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32370, 1/100) overnight at +4°C. The secondary antibody (green) was ab96899, goat anti-rabbit DyLight® 488 (IgG; H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
All lanes: Anti-Bcl-XL antibody [E18] (ab32370) at 1/1000 dilution (purified)

Lane 1: C6 cell lysate
Lane 2: RAW264.7 cell lysate
Lane 3: PC-12 cell lysate
Lane 4: NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Observed band size: 26 kDa why is the actual band size different from the predicted?

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

Flow cytometry analysis of Jurkat cells labelling Bcl-XL with purified ab32370 at 1/20 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
ab32370 (purified) at 1/30 immunoprecipitating Bcl-XL in Jurkat cell lysate (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

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

Anti-Bcl-XL antibody [E18] (ab32370) at 1/500 dilution (unpurified) + whole cell lysate prepared from a clinical sample of breast cancer cells at 20 µg

**Secondary**
Goat anti-rabbit IgG-HRP at 1/1000 dilution

Developed using the ECL technique.

**Observed band size:** 26 kDa why is the actual band size different from the predicted?

**Exposure time:** 15 minutes

Patient recieved anthracycline and taxane neoadjuvant chemotherapy.
Immunohistochemistry of human primary melanoma, staining Bcl-XL (red) with unpurified ab32370. Antigen retrieval was performed in EDTA/Tris buffer (pH 8) before being blocked with 10%NGS for one hour at room temperature. Samples were incubated with primary antibody (1/50) at room temperature for one hour. An AlexaFluor®-conjugated anti-rabbit IgG was used as the secondary antibody.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling Bcl-XL with unpurified ab32370 at 1/50.
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Bcl-XL with purified ab32370 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, a goat anti-rabbit Alexa Fluor® 488 (IgG; 1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/500) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Overlay histogram showing DU145 cells stained with unpurified ab32370 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32370, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG; H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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