abcam

Product datasheet

Anti-Bcl-XL antibody [E18] - BSA and Azide free ab199099



17 References 11 Images

Overview

Product name Anti-Bcl-XL antibody [E18] - BSA and Azide free

Description Rabbit monoclonal [E18] to Bcl-XL - BSA and Azide free

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: Flow Cyt (Intra), ICC/IF, IP, IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Pig 4

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive controlJurkat whole cell lysate (<u>ab7899</u>) can be used as a positive control in WB.

General notes ab 199099 is the carrier-free version of **ab 32370**.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

1

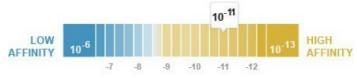
Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 6.50 \times 10^{-11} M$



Learn more about K_D

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number E18 Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab199099 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)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
WB		Use at an assay dependent concentration. Predicted molecular weight: 26 kDa. Please check the parent abID, ab32370 , for more information on dilutions.

Target

Function Potent inhibitor of cell death. Inhibits activation of caspases (By similarity). Appears to regulate

cell death by blocking the voltage-dependent anion channnel (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 modifications

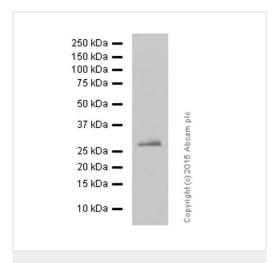
Proteolytically cleaved by caspases during apoptosis. The cleaved protein, lacking the BH4 motif,

has pro-apoptotic activity.

Cellular localization Mitochondrion membrane. Nucleus membrane. Mitochondrial membranes and perinuclear

envelope.

Images



Western blot - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099) + C6 (rat glioma) whole cell lysate at 10 µg

Secondary

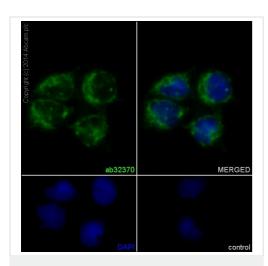
Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 26 kDa

Exposure time: 30 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

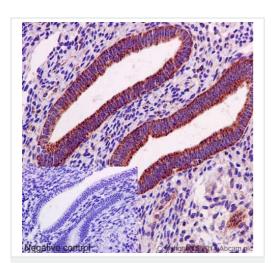


Immunocytochemistry/ Immunofluorescence - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Bcl-XL with purified <u>ab32370</u> at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <u>ab150077</u>, a goat anti-rabbit Alexa Fluor[®] 488 (lgG; 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 lgG (1/500).

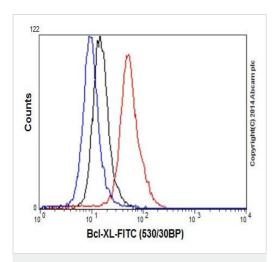
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium tissue labelling BcI-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.

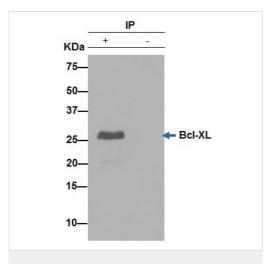
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



Flow Cytometry (Intracellular) - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Intracellular Flow Cytometry analysis of Jurkat cells labelling Bcl-XL with purified <u>ab32370</u> at 1/20 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



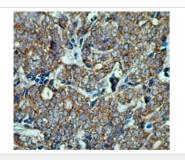
Immunoprecipitation - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

<u>ab32370</u> (purified) at 1/30 immunoprecipitating Bcl-XL in Jurkat cell lysate (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

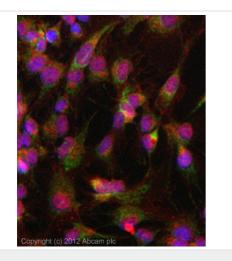
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling Bcl-XL with unpurified <u>ab32370</u> at 1/50.

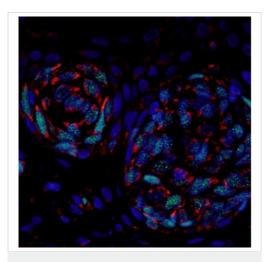
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

ICC/IF image of unpurified <u>ab32370</u> 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 (<u>ab32370</u>, 1/100) overnight at +4°C. The secondary antibody (green) was <u>ab96899</u>, goat <u>anti-rabbit DyLight® 488</u> (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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



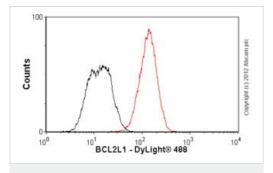
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

Image from Medic S & Ziman MPLoS One. 2010 Apr 22;5(4):e9977. Fig 5.; doi:10.1371/journal.pone.0009977; April 22 2010 PLoS ONE 5(4): e9977.

Immunohistochemistry of human primary melanoma, staining Bcl-XL (red) with unpurified <u>ab32370</u>.

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 lgG was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).

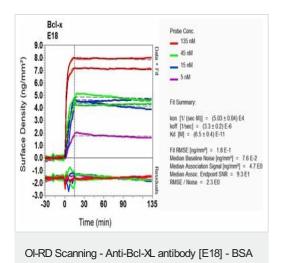


Flow Cytometry (Intracellular) - Anti-Bcl-XL antibody [E18] - BSA and Azide free (ab199099)

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 (lgG; H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was

performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32370).



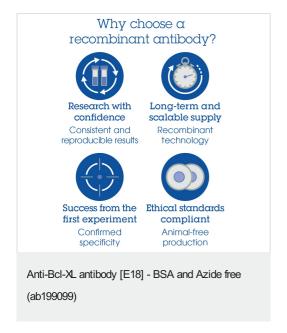
and Azide free (ab199099)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32370</u>).



 $\textbf{Please note:} \ \ \textbf{All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"}$

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