abcam

Product datasheet

Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free ab233694



6 Images

Overview

Product name Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free

Description Rabbit monoclonal [EPR21162] to Bcl-2 (phospho S70) - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, Dot blot, ICC/IF, IP

Species reactivity Reacts with: Human

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

Positive control ICC/IF: HeLa cells.

General notes ab233694 is the carrier-free version of ab218123.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits 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.

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.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR21162

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab233694 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.
WB		Use at an assay dependent concentration. Predicted molecular weight: 26 kDa.
Dot blot		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function Suppresses apoptosis in a variety of cell systems including factor-dependent

lymphohematopoietic and neural cells. Regulates cell death by controlling the mitochondrial membrane permeability. Appears to function in a feedback loop system with caspases. Inhibits caspase activity either by preventing the release of cytochrome c from the mitochondria and/or by binding to the apoptosis-activating factor (APAF-1). May attenuate inflammation by impairing

NLRP1-inflammasome activation, hence CASP1 activation and IL1B release

(PubMed:17418785).

Tissue specificity Expressed in a variety of tissues.

Involvement in disease A chromosomal aberration involving BCL2 has been found in chronic lymphatic leukemia.

Translocation t(14;18)(q32;q21) with immunoglobulin gene regions. BCL2 mutations found in non-Hodgkin lymphomas carrying the chromosomal translocation could be attributed to the lg somatic

hypermutation mechanism resulting in nucleotide transitions.

Sequence similarities Belongs to the Bcl-2 family.

DomainBH1 and BH2 domains are required for the interaction with BAX and for anti-apoptotic activity.

Post-translational modifications

The BH4 motif is required for anti-apoptotic activity and for interaction with RAF1 and EGLN3. The loop between motifs BH4 and BH3 is required for the interaction with NLRP1.

Phosphorylation/dephosphorylation on Ser-70 regulates anti-apoptotic activity. Growth factor-stimulated phosphorylation on Ser-70 by PKC is required for the anti-apoptosis activity and occurs during the G2/M phase of the cell cycle. In the absence of growth factors, BCL2 appears to be phosphorylated by other protein kinases such as ERKs and stress-activated kinases. Phosphorylated by MAPK8/JNK1 at Thr-69, Ser-70 and Ser-87, wich stimulates starvation-induced autophagy. Dephosphorylated by protein phosphatase 2A (PP2A).

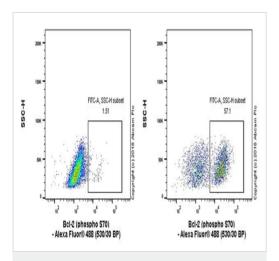
Proteolytically cleaved by caspases during apoptosis. The cleaved protein, lacking the BH4 motif, has pro-apoptotic activity, causes the release of cytochrome c into the cytosol promoting further caspase activity.

Monoubiquitinated by PARK2, leading to increase its stability. Ubiquitinated by SCF(FBXO10), leading to its degradation by the proteasome.

Cellular localization

Mitochondrion outer membrane. Nucleus membrane. Endoplasmic reticulum membrane.

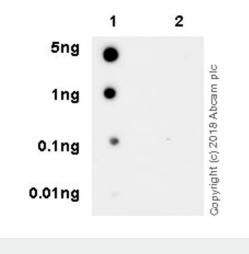
Images



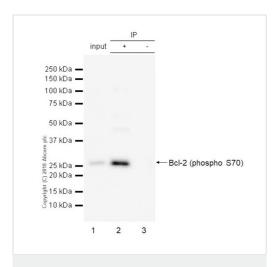
Flow Cytometry (Intracellular) - Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized Jurkat (human T cell leukemia T lymphocyte) treated with 1 μ M paclitaxel for 24h (Right) / Untreated control (Left) cell line labeling Bcl-2 (phospho S70) with <u>ab218123</u> at 1/500 dilution. Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

Square gate shows Bcl-2 (phospho S70) positive signal. The expression profile is consistent with what has been described in the literature (PMID: 10097113)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218123).



Dot Blot - Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694)



Immunoprecipitation - Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694)

Dot blot analysis of Bcl-2 (phospho S70) labeled with <u>ab218123</u> at 1/1000 dilution.

Lane 1: Bcl-2 (phospho S70) peptide.

Lane 2: Bcl-2 non-phospho peptide.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100,000 dilution was used as secondary antibody.

Blocking and dilution buffer: 2% BSA/TBST.

Exposure time: 58 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218123).

Bcl-2 (phospho S70) was immunoprecipitated from 0.35 mg of Jurkat (human T cell leukemia cells from peripheral blood) treated with 1 μ M paclitaxel for 24 hours whole cell lysate with <u>ab218123</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab218123</u> at 0.5 μ g/ml. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/5000 dilution.

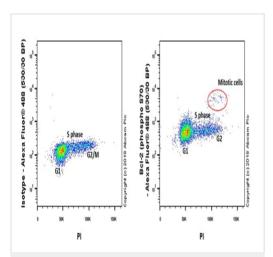
Lane 1: Jurkat treated with 1 μ M paclitaxel for 24 hours whole cell lysate 10 μ g (Input).

Lane 2: <u>ab218123</u> IP in Jurkat treated with 1 μ M paclitaxel for 24 hours whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ (<u>ab172730</u>) instead of <u>ab218123</u> in Jurkat treated with 1 μM paclitaxel for 24 hours whole cell lysate.

Blocking/ Dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 15 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab218123</u>).



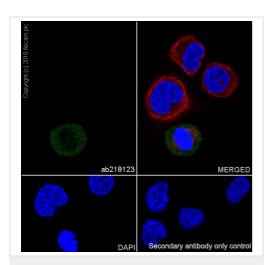
Flow Cytometry (Intracellular) - Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694)

Intracellular flow cytometric analysis of 80% methanol-fixed, 0.1% Tween-20 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Bcl-2 (phospho S70) with **ab218123** at 1/500 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Cells were pretreated with 20 μ g/ml RNase A for 30 minutes to eliminate the non-specific binding between RNA and propidium iodide (PI).

Bcl-2 (phospho S70) is highly expressed in mitotic cells (PMID: 10567572).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218123).



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Bcl-2 (phospho S70) with ab218123 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/100 dilution (green). Confocal image showing positive staining in HeLa cells in M phase. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (<u>ab195889</u>) at 1/200 dilution (red). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218123).



Anti-Bcl-2 (phospho S70) antibody [EPR21162] - BSA and Azide free (ab233694)

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