**Product datasheet**

**Anti-Bim antibody [Y36] ab32158**

![KO VALIDATED Recombinant RabMAb](image)

| ★★★☆☆ 6 Abreviews | 76 References | 12 Images |

**Overview**

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-Bim antibody [Y36]</th>
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<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [Y36] to Bim</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
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<tr>
<td><strong>Specificity</strong></td>
<td>The antibody can recognize all isoforms of Bim. The antibody does not cross-react with other Bcl-2 family members.</td>
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<td><strong>Tested applications</strong></td>
<td>Suitable for: Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF</td>
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<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
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<td><strong>Immunogen</strong></td>
<td>Synthetic peptide within Human Bim aa 1-100. The exact sequence is proprietary. (Peptide available as ab179844)</td>
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<td><strong>Positive control</strong></td>
<td>WB: Raji, Jurkat, A431, Molt-4, A20, MEF, Raw264.7 and PC-12 cell lysate; Human and mouse thymus, mouse and rat spleen tissue lysate. IHC: breast carcinoma tissue. ICC/IF: A20 and Raji cells. Flow Cyt (intra): A431 and Raji whole cell lysate. HAP1-WT cells. IP: Raji whole cell lysate.</td>
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<tr>
<td><strong>General notes</strong></td>
<td><strong>Mouse and Rat species are recommended based on WB results, we do not guarantee IHC-P for Mouse and Rat.</strong></td>
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<td>This product is a recombinant monoclonal antibody, which offers several advantages including:</td>
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<td>- High batch-to-batch consistency and reproducibility</td>
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<tr>
<td></td>
<td>- Improved sensitivity and specificity</td>
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<tr>
<td></td>
<td>- Long-term security of supply</td>
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<td>- Animal-free production</td>
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<td>For more information see here.</td>
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<tr>
<td></td>
<td>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.</td>
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</table>

**Properties**

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
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<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.</td>
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<tr>
<td><strong>pH</strong></td>
<td>7.20</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.01% Sodium azide</td>
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<tr>
<td><strong>Constituents</strong></td>
<td>49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA</td>
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</tbody>
</table>
Purity: Protein A purified
Clonality: Monoclonal
Clone number: Y36
Isotype: IgG

Function:
Induces apoptosis. Isoform BimL is more potent than isoform BimEL. Isoform Bim-alpha1, isoform Bim-alpha2 and isoform Bim-alpha3 induce apoptosis, although less potent than the isoforms BimEL, BimL and BimS. Isoform Bim-gamma induces apoptosis.

Tissue specificity:
Isoform BimEL, isoform BimL and isoform BimS are the predominant isoforms and are ubiquitously expressed with a tissue-specific variation. Isoform Bim-gamma is most abundantly expressed in small intestine and colon, and in lower levels in spleen, prostate, testis, heart, liver and kidney.

Sequence similarities:
Belongs to the Bcl-2 family.

Domain:
The BH3 motif is required for Bcl-2 binding and cytotoxicity.

Cellular localization:
Mitochondrion and Endomembrane system. Associated with intracytoplasmic membranes.

Applications

The Abpromise guarantee: Our Abpromise guarantee covers the use of ab32158 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>Flow Cyt (Intra)</td>
<td></td>
<td>1/50 - 1/100.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. Mouse and Rat species are recommended based on WB results, we do not guarantee IHC-P for Mouse and Rat.</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/40 - 1/50.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100 - 1/250.</td>
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</tbody>
</table>

Target

Function:
Induces apoptosis. Isoform BimL is more potent than isoform BimEL. Isoform Bim-alpha1, isoform Bim-alpha2 and isoform Bim-alpha3 induce apoptosis, although less potent than the isoforms BimEL, BimL and BimS. Isoform Bim-gamma induces apoptosis.

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Sequence similarities:
Belongs to the Bcl-2 family.

Domain:
The BH3 motif is required for Bcl-2 binding and cytotoxicity.

Cellular localization:
Mitochondrion and Endomembrane system. Associated with intracytoplasmic membranes.
Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Bim knockout HAP1 whole cell lysate (20 µg)
Lane 3: Raji whole cell lysate (20 µg)
Lane 4: Jurkat whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32158 observed at 25 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab32158 was shown to specifically react with Bim when Bim knockout samples were used. Wild-type and Bim knockout samples were subjected to SDS-PAGE. ab32158 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.

Overlay histogram showing HAP1 wildtype (green line) and HAP1-BCL2L11 knockout cells (red line) stained with ab32158. The cells were fixed 80% methanol (5 min), and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32158, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG1 isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-BCL2L11 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.
Immunocytochemistry/Immunofluorescence analysis of Raji (Human Burkitt’s lymphoma cell line) labeling Bim with ab32158 at a dilution of 1/250. Cells were fixed with 100% methanol. Ab150077 (1/1000) was used as the secondary antibody. Cells were co-stained with ab7291, a mouse anti-tubulin antibody (1/200) using ab150120 as the secondary. Nuclei were counterstained with DAPI (blue).

Secondary antibody only control, cells without incubation with the primary antibody was used as negative control.

Confocal image showing cytoplasmic staining on Raji cell line

ab32158 staining Bim in human breast cancer tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Antigen retrieval was by heat mediated antigen retrieval using Tris/EDTA Buffer, PH9 (ab93684). Samples were incubated with primary antibody (1/100 in blocking buffer) and a Biotin-conjugated Donkey anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody. Cytoplasmic staining can be seen in the human breast cancer cells. Hematoxylin was used as a counter stain.
Intracellular Flow Cytometry analysis of Raji (human Burkitt's lymphoma) whole cell lysate labeling Bim with ab32158 at 1/100 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

Ab32158 at 1/50 immunoprecipitating Bim in Raji (human Burkitt's lymphoma) whole cell lysate.

Lane 1 (input): Raji whole cell lysate (10µg)
Lane 2 (+): ab32158 + Raji whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32158 in Raji whole cell lysate.

For western blotting, ab32158 (1/1000) was used as the primary antibody and ab131366 VeriBlot for IP Detection Reagent (HRP) was used for detection (1/10 000).

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.
**Western blot - Anti-Bim antibody [Y36] (ab32158)**

**All lanes**: Anti-Bim antibody [Y36] (ab32158) at 1/2000 dilution

**Lane 1**: Raji (human Burkitt's lymphoma) whole cell lysate
**Lane 2**: A431 (human epidermoid carcinoma) whole cell lysate
**Lane 3**: Molt-4 (human acute lymphoblastic leukemia) whole cell lysate
**Lane 4**: Human thymus tissue lysate
**Lane 5**: Mouse thymus tissue lysate
**Lane 6**: A20 (mouse reticulum cell sarcoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size**: 22 kDa

**Observed band size**: 22, 18 kDa

**Exposure time**: Lane 1-5: 3 minutes; Lane 6: 2 seconds

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

The observed molecular weight is consistent with the literature (PMID: 24872388)
Immunocytochemistry/Immunofluorescence analysis of A20 (Mouse reticulum sarcoma cell line) labeling Bim with ab32158 at a dilution of 1/250. Cells were fixed with 100% methanol. Ab150077 (1/1000) was used as the secondary antibody. Cells were co-stained with ab7291, a mouse anti-tubulin antibody (1/200) using ab150120 as the secondary. Nuclei were counterstained with DAPI (blue).

Secondary antibody only control, cells without incubation with the primary antibody was used as negative control.

Confocal image showing cytoplasmic staining on A20 cell line

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labelling Bim with ab32158 at 1/50 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

All lanes : Anti-Bim antibody [Y36] (ab32158) at 1/2000 dilution

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Rat spleen tissue lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 4 : Raw264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution
Predicted band size: 22 kDa

Observed band size: 22, 18 kDa

Exposure time: Lane 1-3: 3 minutes; Lane 4: 10 seconds

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

The observed molecular weight is consistent with the literature (PMID: 24872388)

Anti-Bim antibody [Y36] (ab32158) at 1/500 dilution + Whole cell lysates prepared from human jurkat cells at 200000 cells

Secondary
HRP conjugated Donkey polyclonal to rabbit IgG at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 22 kDa

Observed band size: 22 kDa

Exposure time: 30 seconds

Primary diluted in PBS (5% BSA + 0.1% tween20) and incubated with sample for 1 hour and 30 minutes at 20°C.

Why choose a recombinant antibody?
- Research with confidence: Consistent and reproducible results
- Long-term and scalable supply: Recombinant technology
- Success from the first experiment: Confirmed specificity
- Ethical standards compliant: Animal-free production

Anti-Bim antibody [Y36] (ab32158)
Please note: All products are “FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES”

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