

ORDERING INFORMATION

Catalog Number: MAB851

Clone: 78616

Lot Number: COY02

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

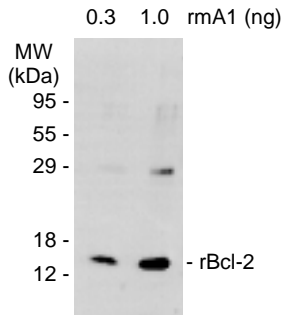
Reconstitution: sterile PBS

Specificity: mouse A1

Immunogen: *E. coli*-derived rmA1 (amino acid residues 1 - 152)

Ig class: rat IgG_{2A}

Recommended Application:
Western blot



Immunoblots of 0.3 ng and 1 ng of recombinant mouse A1, amino acids 1 - 152 (recombinant A1 is missing the carboxy terminal mitochondria targeting sequence). Samples were electrophoresed on 15% gels and immunoblotting was with 1.0 µg/mL anti-A1. A one minute exposure is shown.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived, recombinant mouse Bcl-2 related protein A1 (rmA1) (amino acid residues 1 - 152). The IgG fraction of ascites fluid was purified by Protein G affinity chromatography. A1 is an anti-apoptotic outer mitochondrial membrane protein that prevents release of cytochrome c from the mitochondria intermembrane space into the cytosol. A1 prevents apoptosis by binding to the BH3 region of pro-apoptotic Bcl-2 family members.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in 100 µL of PBS containing 0.02% Na₂S₂O₃.

Storage

Avoid repeated freezing and thawing by aliquoting smaller portions of the reconstituted antibody into Eppendorf tubes and storing at -20° C.

Specificity

The antibody detects mouse Bcl-2 related protein A1.

Application

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Western blotting

<u>Blotting buffer</u>	<u>Blocking solution</u>	<u>Antibody solution</u>
25 mM Tris, pH 7.5	2% nonfat dry milk in blotting buffer	1% nonfat dry milk in blotting buffer
0.15 M NaCl	pH to 7.5	pH to 7.5
0.05% Tween 20		

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.
2. Incubate the membrane overnight in antibody solution containing 1.0 µg/mL rat anti-mouse A1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in antibody solution containing a 1:2,000 dilution of HRP-conjugated goat anti-rat (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer and visualize the immunodetected bands using the ECL system.
6. Detection was with ECL Procedure (Amersham).

Cell lysates for western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Tissue extracts for western blotting: To prepare tissue extracts, tissue is excised, rinsed with cold PBS, minced and homogenized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and the extract is heated in a boiling water bath for 3 - 5 minutes. The extract is centrifuged at 12,000 x g to remove insoluble material.