

ORDERING INFORMATION

Catalog Number: MAB828

Clone: 542808

Lot Number: CCWH01

Size: 100 µg

Storage: -20° C

Specificity: human Mcl-1

Immunogen: *E. coli*-derived rhMcl-1

Ig Class: mouse IgG_{2b}

Recommended Applications:

Western blot
Immunohistochemistry

Background

Mcl-1 (myeloid cell leukemia-1; also known as Bcl-2-like protein 3) is a member of the Bcl-2 family of proteins. Alternative splicing creates two distinct isoforms: 40 kDa Mcl-1L (long; 350 amino acids (aa)) enhances cell survival by inhibiting apoptosis, while 32 kDa Mcl-1S (short; 271 aa with divergence in the last 41 aa) promotes apoptosis. The elimination of Mcl-1L is a required step for DNA damage-induced apoptosis. Mcl-1 can be modified by phosphorylation on S121 and T163 by JNK, which triggers apoptosis, or polyubiquitination, which enhances degradation of Mcl-1. Within the first 230 aa, human Mcl-1 shares ~68% aa identity with mouse and rat Mcl-1.

Preparation

This antibody was produced using a hybridoma elicited from a mouse immunized with purified, *E. coli*-derived recombinant human myeloid cell leukemia-1 (rhMcl-1; aa 1 - 230; Accession # Q07820). The IgG fraction of the hybridoma culture supernatant was purified by protein G chromatography.

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% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human Mcl-1 at 38 kDa on Western blot.

Applications

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

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween[®] 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

Antibody Solution

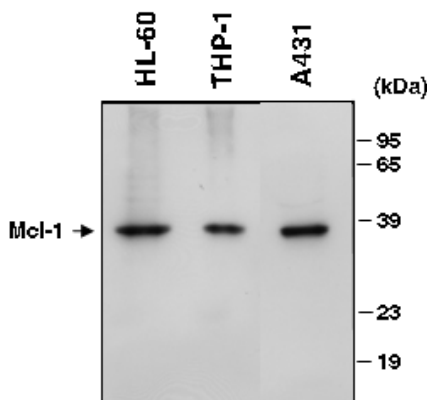
2% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 µg/mL MAB828.
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 at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent substrate detection system according to manufacturers protocol.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 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.

Immunohistochemistry - This antibody was used at a concentration of 25 µg/mL with appropriate secondary reagents to detect Mcl-1 in paraffin-embedded human lymphoma tissue sections. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by each laboratory for each application.



Detection of Mcl-1 with MAB828.

Lysates from human HL-60, THP-1 and A431 cells were resolved by SDS-PAGE, transferred to Immobilon membranes and immunoblotted with 1.0 µg/mL MAB828, as described in *Protocols for Immunoblotting*.