

## 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<sub>2b</sub>

**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% NaN<sub>3</sub>.

## 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

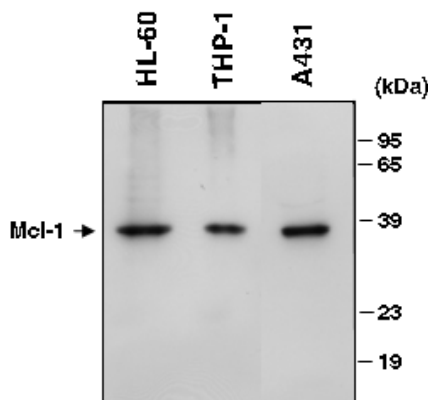
Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween <sup>®</sup> 20	Adjust pH to 7.4	Adjust pH to 7.4

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 x 10<sup>6</sup> - 1 x 10<sup>7</sup> 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*.