

# TREVIGEN™ Product Data

For Research Use Only. Not For Use In Diagnostic Procedures.

## Anti-Mcl-1 Polyclonal Antibody

Catalog #: 2316-PC-075

Size: 75 µg

**Description:** Rabbits were immunized with the KLH-coupled, synthetic peptide RQSLEIISRYLREQATGAKDC (corresponding to amino acids 175-194 of human Mcl-1). Cysteine was added to the carboxyl-terminal for coupling to KLH and for coupling to an affinity matrix. The murine Mcl-1 differs by a single amino acid substitution of S for A at position 192 of human Mcl-1. Polyclonal antibody was affinity-purified on a column derivatized with the peptide.

**Physical State:** This antibody is provided in phosphate-buffered saline (PBS) without preservative. The final antibody concentration is 1 µg/µl.

**Endotoxin Level:** < 10 ng per 1 mg of the antibody as determined by the LAL method.

**Immunogen:** Amino acids 175 - 194 of human Mcl-1.

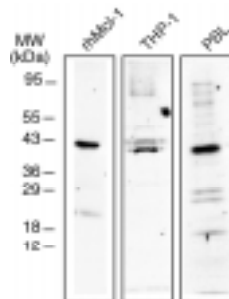
**Ig Class:** Mcl-1 specific rabbit IgG.

**Specificity:** The antibody detects human Mcl-1. The antibody also detects a 27 - 34 kDa band in extracts of mouse L929 and DA3 cells. This band may be the murine form of Mcl-1.

**Storage:** The antibody can be stored at 2°C to 4°C for at least 1 month, or can be aliquoted and stored frozen at -20°C to -80°C for at least six months without detectable loss of activity. Avoid repeated freezing and thawing by aliquoting smaller portions of the antibody into Microtubes and storing at -20°C.

**Applications:** Western blotting  
An antibody concentration of 1.5 µg/ml is recommended.

Immunoblots of SDS-extracts from  $2 \times 10^5$  human peripheral blood lymphocytes (PBL) and  $2 \times 10^5$  human THP-1 cells. SDS extracts of *E. coli* cells expressing human Mcl-1 were also blotted. Extracts were electrophoresed on 15% gels and immunoblotting was with 1.5 µg/ml anti-Mcl-1. Incubation with anti-Mcl-1 was overnight at 4°C and detection was by chemiluminescence. Exposures shown for rhMcl-1 and THP-1 are 30 seconds and the PBL exposure is 1 minute.



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## Procedure for Immunoblotting:

Blotting buffer: 25 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween®20.

Blocking solution: 5% nonfat dry milk in blotting buffer, pH to 7.5.

Antibody solution: 2% nonfat dry milk in blotting buffer, pH to 7.5.

Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.

Incubate the membrane overnight at 4°C in antibody solution containing 1.5 µg/ml rabbit anti-Mcl-1.

Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing membrane containers often reduces background.

Incubate the membrane at room temperature in antibody solution containing a 1:2,000 dilution of HRP-conjugated Protein A (Amersham).

Wash the membrane for 1 hour with 5 or more changes of blotting buffer.

## Cell Lysates for Western Blotting:

To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 250 mM Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  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.

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Catalog #: 2316-PC-075

Lot #: 2895B0

Storage: -20°C to -80°C

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