

TREVIGEN® Product Data

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

Anti-human/mouse BIM Polyclonal Antibody

Catalog #: 2341-PC-050

Size: 50 µg

Description: Rabbits were immunized with the KLH-coupled synthetic peptide AERPPQLRPGAPTSLQTEC corresponding to amino acids 22 - 39 of human and mouse BIM. Cysteine was added to the carboxyl-terminal for coupling to KLH and for coupling to an affinity matrix.

Physical State: Affinity purified antibody at 1 mg/ml provided in phosphate buffered saline without preservative.

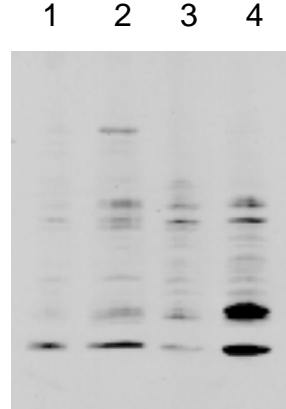
Specificity: This antibody detects human and mouse BIM.

Storage: Store at -20 °C. To avoid repeat freeze/thaws, freeze in working aliquots at -20°C.

Applications: Western blot.

For Western blot analysis, the recommended concentration is 1 µg/ml but empirical determination will be required for optimal results. See reverse for immunoblotting protocol.

Immunoblots of SDS-extracts from 2×10^5 human Jurkat cells (1), human peripheral blood lymphocytes (2), mouse CTLL-2 cells (3) and mouse spleen (4). Tissue was excised, rinsed in cold PBS, minced, and homogenized in hot 2X SDS sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris (pH 6.8), 10% glycerol, 0.01 % bromophenol blue) at $2 \times 10^6 - 1 \times 10^7$ cells/ml. The extracts were heated in a boiling water bath for 5 minutes followed by centrifugation at 12,000 x g to remove insoluble material. Cells were solubilized in hot 2X SDS sample buffer at $2 \times 10^6 - 1 \times 10^7$ cells/ml. The extracts were heated in a boiling water bath for 5 minutes followed by sonication with a probe sonicator with 3 - 4 second bursts of 5 - 10 seconds each. Cell and tissue extracts were diluted in 1X SDS sample buffer and electrophoresed on a 20% SDS-polyacrylamide gel and transferred to PVDF membrane. Membranes were incubated with 1 µg/ml anti-BIM overnight at 4 °C, followed by detection using a peroxidase conjugated Protein A and chemiluminescence.



Procedure for Immunoblotting using Peroxidase Detection:

Transfer the electrophoresed proteins to nitrocellulose or PVDF membrane by Western transfer. Incubate the membrane for 1 hour at room temperature in 2% (w/v) nonfat dry milk in 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20.

Incubate the membrane overnight at 4°C in 1:1000 of antibody in 1% (w/v) nonfat dry milk in 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20. Empirical determination of primary antibody concentration will be required for optimal results.

Wash the membrane at room temperature for at least 15 minutes with 3 changes of 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20.

Incubate the membrane at room temperature for 1 hour in 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20 containing a dilution of Protein A conjugated to Horseradish peroxidase. Empirical determination of the secondary conjugate will be required for optimal results.

Wash the membrane for at least 15 minutes with 3 changes of 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20, then rinse in water.

Develop peroxidase reaction using Trevigen's Blue Membrane Solution (Cat# 4857-20-13) or Peroxyglow chemiluminescence reagents (Cat#s 4855-20-13 and 4855-20-14).

Tween 20 is a registered trademark of ICI Americas, Inc., Wilmington, DE

Lot specific information:

Lot#:

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BIM

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Storage: - 20°C

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