

# TREVIGEN® Product Data

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

## Anti-human APAF-1 Monoclonal Antibody

**Catalog #:** 2340-MC-050

**Size:** 50 µg

**Description:** This monoclonal antibody, clone 94408.11, was produced from a murine hybridoma elicited from a mouse immunized with a recombinant fragment of human APAF-1 corresponding to amino acids 10-214 (accession number AF013263).

**Physical State:** Protein G affinity purified antibody at 1 mg/ml provided in phosphate buffered saline without preservative.

**Specificity:** This antibody detects human APAF-1.

**Ig class:** Mouse IgG<sub>2b</sub>

**Storage:** Store at -20°C. To avoid repeated 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.

1 2



Immunoblots of SDS-extracts from  $2 \times 10^6$  (lane 1) and  $7 \times 10^4$  (lane 2) human Jurkat cells. Cells were solubilized 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 sonication with a probe sonicator with 3 - 4 second bursts of 5 - 10 seconds each. Cell extract was diluted in 1X SDS sample buffer and electrophoresed on a 5-15% gradient SDS-polyacrylamide gel and transferred to PVDF membrane. Membranes were incubated with 1 µg/ml anti-APAF-1 overnight at 4 °C, followed by detection using a peroxidase conjugated secondary antibody 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 overnight at 4 °C in 25 mM Tris (pH 7.5), 0.15 M NaCl, 0.05% Tween 20 containing a dilution of anti-mouse antibody conjugated to Horseradish peroxidase. Empirical determination of secondary antibody concentration 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#:

© 2001 Trevigen, Inc. All Rights Reserved. Trevigen is a registered trademark of Trevigen, Inc.

v20403

# TREVIGEN®

8405 Helgerman Court, Gaithersburg, MD 20877 USA

Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800

Fax: 301-216-2801 • e-mail: [info@trevigen.com](mailto:info@trevigen.com) • [www.trevigen.com](http://www.trevigen.com)

**Anti-Human**

**APAF-1**

**Monoclonal Antibody**

**Catalog #: 2340-MC-050**

**Storage: - 20°C**

# TREVIGEN®

**1-800-873-8443**