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

Anti-Human/Mouse Caspase 2 Polyclonal Antibody

Catalog #: 2304-PC-040 **Size:** 40 µg

Description: Rabbits were immunized with the KLH-coupled synthetic peptide KEREGYAPGTEFHRC corresponding to amino acids 414 - 428 of mouse Caspase 2.

Physical State: Affinity purified antibody at 0.8 mg/ml provided in phosphate buffered saline without preservative.

Specificity: This antibody detects human and mouse procaspase 2 and and the small caspase 2 subunit that is generated during proteolytic cleavage.

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 0.8 µg/ml, but empirical determination will be required for optimal results. See reverse for immunoblotting protocol.

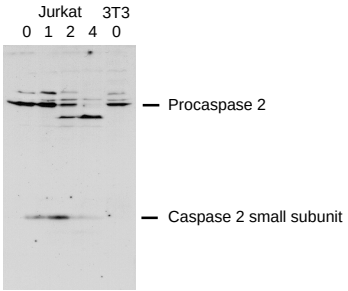

Fig.1 Immunoblots of SDS-extracts from 4 x 10^5 human Jurkat cells treated for 0, 1, 2, or 4 hours with 1 µm staurosporine, and from 2 x 10^5 mouse 3T3 cells. Cells were solubilized in hot 2X SDS sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris (pH 6.8), 10% glycerol, 0.01 % bromophenol blue) at 2 x 10^6 - 1 x 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 extracts were diluted in 1X SDS sample buffer and were electrophoresed on a 15% SDS-polyacrylamide gel and transferred to PVDF membrane. Membranes were incubated with 0.8 µg/ml anti-caspase 2 overnight at 4 ˚C, followed by detection using 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 60 minutes with 5 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 secondary conjugate concentration will be required for optimal results.

Wash the membrane for 60 minutes with 5 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).

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