

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-Cleaved Caspase-3 Rabbit Polyclonal Antibody

Catalog #: 2305-PC-100

Size: 100 µl

Description: Rabbits were immunized with the KLH-coupled synthetic peptide CRGTELDGCIETD. The polyclonal antibody was affinity purified on a column derivatized with the peptide. It detects the processed large subunit (p18 fragment) but not the unprocessed, zymogen form of caspase-3. In apoptotic lysates, the antibody may recognize the induction of a strong p30 band (Fig. 1).

Physical State: This antibody is provided as affinity purified IgG fraction in 1X PBS containing 1 mg/ml BSA, 50% glycerol, and 0.01% sodium azide. The final antibody concentration is 0.1 mg/ml.

Immunogen: 13-amino acid peptide sequence from carboxyl terminus of the human and mouse caspase-3 p18 subunit.

Specificity: The antibody detects human and mouse cleaved caspase-3.

Storage: Store at -20° C.

Applications: For immunodetection, an antibody dilution of 1:500-1:1000 is recommended. May also be used for immunoprecipitation and immunocytochemistry at a 1:100-1:1000 dilution. Empirical determination will be required for optimal results.

Reference: 1. Srinivasan, A., K.A. Roth, R.O. Sayers, K.S. Shindler, A.M. Wong, L.C. Fritz, and K.J. Tomaselli. 1998. *In situ* immunodetection of activated caspase-3 in apoptotic neurons in the developing nervous system. *Cell Death Diff* 5:1004-1016.

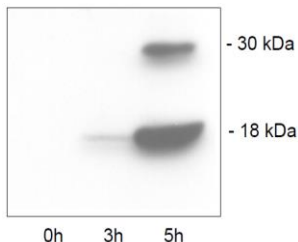


Fig 1. Immunoblot of SDS-extracts from mouse cells treated with 25 µM etoposide. Samples were electrophoresed on a 12% Tris-Glycine gel and blotted onto a PVDF membrane. The 18 kDa cleaved caspase-3 was detected by Trevigen's anti-cleaved caspase-3 followed by anti-rabbit conjugated to horseradish peroxidase and chemiluminescence.

TREVIGEN®

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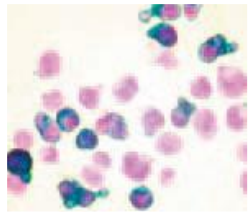


Fig. 2. Jurkat cells were treated with 25 μ M etoposide for 5 hours to induce apoptosis. Cells were labeled with 1:250 dilution of anti-cleaved caspase-3 followed by anti-rabbit horseradish peroxidase. Detection was per-formed with Trevigen's Blue Label and counterstained with Nuclear Fast Red.

Cell Lysates for Western Blotting:

To prepare total cell lysates, cells are solubilized in 1X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 250 mM Tris, pH 6.8, 10% glycerol, and bromophenol blue) at 5×10^5 - 1×10^6 cells per ml. The extracts are heated in a boiling water bath for 5 minutes. Electrophoresis on 12% Tris-Glycine SDS-PAGE gels.

Procedure for Immunoblotting using Peroxidase Detection:

Blotting buffer: 12 mM Tris base, 96 mM Glycine, and 20% MeOH.

Blocking solution: 5% (w/v) nonfat dry milk in PBS.

Antibody solution: 5% (w/v) nonfat dry milk, 0.05% Tween in PBS.

- Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1/2 hour at room temperature in blocking solution.
- Incubate the membrane overnight at 4°C in antibody solution containing a 1:500 - 1:1000 dilution of cleaved caspase-3 rabbit polyclonal antibody. Empirical determination of primary antibody concentration will be required for optimal results.
- Wash the membrane at room temperature for 15 minutes with 3 changes of 0.05% Tween in PBS. Changing membrane containers often reduces background.
- Incubate the membrane at room temperature for 1 hour in an antibody solution containing anti-rabbit IgG conjugated to horseradish peroxidase. Empirical determination of secondary antibody concentration will be required for optimal results.
- Wash the membrane for 15 minutes with 3 changes of 0.05% Tween in PBS.
- Develop peroxidase reaction using e.g. chemiluminescence.

Related Products:

Catalog #	Description	Size
4411-PC-100	Anti-Phosphorylated Histone H2AX polyclonal	100 μ l
6370-MC-100	Anti-human/murine-Cytochrome C	100 μ g
6380-MC-100	Anti-human/murine-Holocytochrome C	100 μ g
2291-MC-100	Anti-human-Bcl-2 mAb (clone YTH-8C8)	100 μ g
2290-MC-100	Anti-Bcl-2 mAb (clone YTH-10C4)	100 μ g
2281-MC-100	Anti-human-Bax mAb (clone YTH-6A7)	100 μ g
6361-PC-100	Anti-human/mouse-PBR polyclonal	100 μ l
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 μ l
4336-BPC-100	Anti- PAR polymer polyclonal	100 μ l
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 μ g

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