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Certificate of Analysis

Anti-Caspase-3

(rabbit polyclonal IgG)
Catalog # 06-735
Lot # R0702B16

Immunogen: Human full-length caspase-3 fusion protein containing a histidine-6 tag.

Specificity: Recognizes full-length caspase-3 and proteolytic fragments.

Species Cross-reactivity: Human, mouse and rat.

Quantity and Formulation: **200 µg** protein A purified IgG in **200 µL** of 0.07M Tris-glycine, 0.105M NaCl, pH 7.4, 0.035% sodium azide, 30% glycerol. Cold liquid.

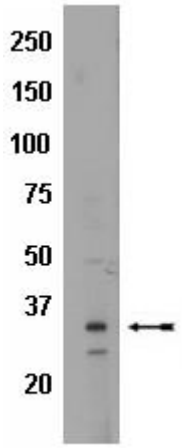
Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 1 µg/mL of this lot detected pro-Apopain (32 kDa) in RIPA lysates from human non-stimulated A431, mouse 3T3 and rat PC12 cells.

Included Positive Antigen Control for Western Immunoblot Analysis: Catalog # 12-301, non-simulated A431 cell lysate. Use 20µg per lane for minigels.



Immunoblot Analysis
Non-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Caspase-3 (1 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates pro-Apopain (32kDa).

Background: Caspase-3/Yama/Apopain, also known as proICE and CPP32, is a member of the ICE-like protease family. Caspase-3 is expressed as a 32 kDa precursor, which is proteolytically cleaved to yield an active form comprised of 17kDa and 12kDa subunits, respectively. The active enzyme appears to play an important role in the regulation or execution of apoptosis. One of its target substrates is the enzyme poly (ADP-ribose) polymerase (PARP) which is involved in DNA repair and maintenance of genome integrity.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1 µg/mL of anti–Caspase-3**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (**a goat anti-rabbit IgG 1:1000 dilution** was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used). Detection may require long exposure times (10-30 min.) in some systems.