

**Anti-Caspase 3**  
(rabbit polyclonal IgG)  
Catalog # 06-735  
Lot # 18079

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

**Specificity:** Recognizes full-length Caspase 3 at 32kDa and proteolytic fragments as demonstrated after *in vitro* cleavage of recombinant Caspase 3.

**Species Cross-reactivity:** Human, mouse and rat.

**Formulation:** 200µg protein A purified IgG in 200µl of 0.07M Tris-glycine, pH 7.4, 0.105M NaCl, 0.035% sodium azide with 30% glycerol. Liquid at -20°C.

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

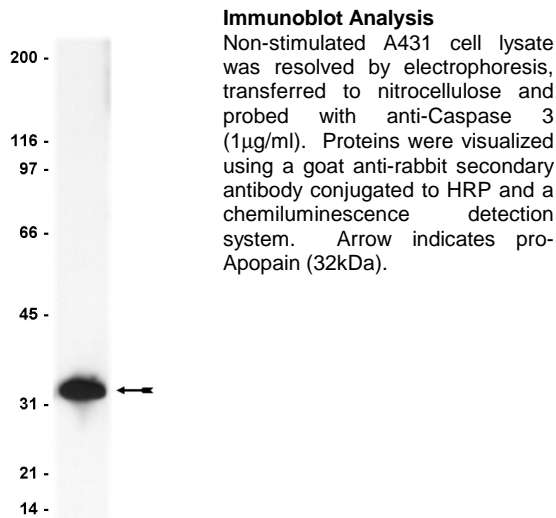
**FOR RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS**

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**Quality Control Testing**

**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected pro-Apopain (32kDa) in RIPA lysates from non-stimulated human A431 cells; previous lots detected Caspase 3 in RIPA lysates of mouse 3T3 and rat PC12 cells.

**Included Positive Antigen Control:** Catalog # 12-301, non-stimulated A431 cell lysate. Use 20µg per lane for minigels.



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**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 32kDa 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<sub>3</sub>VO<sub>4</sub>; 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 (MLK) for 60 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/ml of a-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 a-rabbit** HRP conjugated IgG, 1:3000 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.