

## Certificate of Analysis

**Anti-Caspase 3**  
(rabbit polyclonal IgG)  
Catalog # 06-735  
Lot # DAM1576927

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

**Specificity:** Recognizes full-length Caspase 3 (Yama/Apopain), Mr 32kDa and proteolytic fragments as demonstrated after *in vitro* cleavage of recombinant Caspase 3 and immunoblotting with apoptotically induced HL-60 cells treated with peroxyinitrite.

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

**Formulation:** 200µg protein A purified IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide. Liquid at 2-8°C.

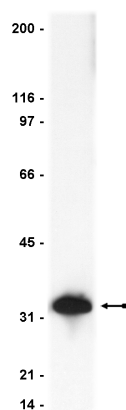
**Storage and Stability:** Stable for 2 years at 2-8°C from date of shipment. For maximum recovery of product, centrifuge the original vial prior to removing the cap.

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

### Quality Control Testing

**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected the 32kDa precursor of Caspase 3 in RIPA lysates from non-stimulated human A431 cells. Previous lots detected Caspase 3 in RIPA lysates of mouse 3T3/A31 and rat PC12 cells.

**Included Positive Antigen Control:** Catalog # 12-301, non stimulated A431 lysate. **Add 2.5µl of 2-mercaptoethanol per 100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.



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

### Application Reference:

Martini, J., *et al.*, Mol. Endocrinol. **14:** 1536-1549, 2000.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans or animals.

### 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 EDTA; 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 (Catalog # 20-200), (PBS-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/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** HRP conjugated IgG, Catalog # 12-348, 1:5000 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 minutes) in some systems.

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