



cell signaling solutions

## Certificate of Analysis

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### Anti-Caspase 3, clone 4-1-18

(mouse monoclonal IgG<sub>2a</sub>)

Catalog # 05-654

Lot # 22206

**Immunogen:** N-terminal 6HIS tagged fusion protein corresponding to full length human Caspase 3. Clone 4-1-18.

**Specificity:** Recognizes full-length Caspase 3 at Mr 32kDa and 17kDa subunit of 10ng of recombinant Caspase 3, Catalog # 14-264.

**Species Cross-reactivity:** Human, not dog.

**Formulation:** 100µg of protein G purified mouse IgG<sub>2a</sub> in 100µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

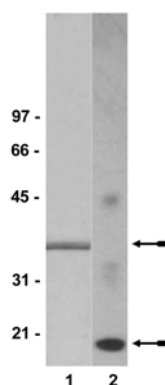
**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

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

### Quality Control Testing

**Immunoblot Analysis:** 2.0µg/ml of this lot detected Caspase 3 in RIPA lysates from Jurkat cells.

**Included Positive Antigen Control:** Catalog # 12-303, Jurkat lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.



#### Immunoblot Analysis

Jurkat cell lysate (lane 1) or recombinant Caspase 3, Catalog 14-264, (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Caspase 3 (2µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Caspase 3 (~32kDa) and 17kDa subunit.

### General References:

1. Fearnhead, H.O., *et al.*, *Proc. Natl. Acad. Sci. USA* **95**: 13664-13669, 1998.
2. Faleiro, L., and Lazebnik, Y., *The Journal of Cell Biology* **151**: 951-959, 2000.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml each 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 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2.0 $\mu$ g/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-mouse HRP conjugated IgG, 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).