

## Certificate of Analysis

**Anti-Caspase 9, clone 96-2-22**  
(mouse monoclonal IgG)  
Catalog # 05-572  
Lot # DAM1647092

**Immunogen:** The N-terminal fragment of human Caspase 9 (amino acid residues 1-134). Clone 96-2-22.

**Specificity:** Recognizes the proform and the active cleaved form of Caspase 9, Mr 46kDa and 34kDa, respectively. Does not recognize Caspase 3, 6, 7 and 8.

**Species Cross-reactivity:** Human **not** mouse or rat.

**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.

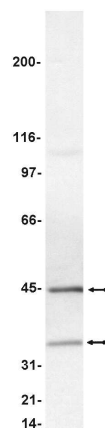
**Formulation:** 100µg of protein G purified mouse IgG in 100µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide. Frozen at -20°C.

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

### Quality Control Testing

**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected the proform of Caspase 9 in RIPA lysates from both A431 and Raji cells. 0.5-2µg/ml of a previous lot detected the proform and cleaved Caspase 9 in RIPA lysates from HFF cells.

**Included Positive Antigen Control:** Catalog # 12-301, non-stimulated A431 cell 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

Representative lot data. A431 cell lysate was resolved by electrophoresis, transferred to PVDF and probed with anti-Caspase 9 (2 µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicate the proform and cleaved Caspase 9 (~46kDa and ~34kDa).

### Application References:

Fearnhead, H.O., *et al.*, *Proc. Natl. Acad. Sci. USA* **95**: 13664-13669, 1998.  
Rodriguez, J. and Y. Lazebnik, *et al.*, *Genes Dev.* **13**: 3179-3184, 1999.

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### 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 PVDF. Wash the blotted PVDF twice with water.
2. Block the blotted PVDF in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the PVDF with **0.5-2 $\mu$ g/ml of anti-Caspase 9**, diluted in freshly prepared PBS-MLK overnight with agitation at 4 $^{\circ}$ C.
4. Wash the PVDF twice with water.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the PVDF with water twice.
7. Wash the PVDF in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the PVDF in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

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