

Certificate of Analysis

Anti-Caspase 7, clone 7-1-11

(mouse monoclonal IgG)

Catalog # 05-578

Lot # 20139

Immunogen: GST fusion protein corresponding to residues 1-303 of human Caspase 7. Clone 7-1-11.

Specificity: Recognizes Caspase 7, Mr 37kDa. Previously shown to recognize cleaved Caspase 7, Mr 19kDa¹.

Species Cross-reactivity: Human. Other species not tested.

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: 100mg of protein G purified IgG in 100ml 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.

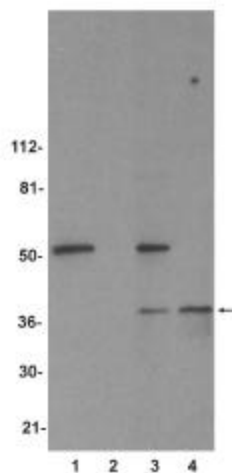
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2.0µg/ml of this lot detected Caspase 7 in RIPA lysates from Jurkat cells.

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

Immunoprecipitation: 4µg of this lot immunoprecipitated Caspase 7 from 500µg of Jurkat RIPA lysate.



Immunoprecipitation/Immunoblot Analysis

Lanes 1 and 3: Jurkat cell lysate (0.5mg) was immunoprecipitated with 4µg of anti-Caspase 7. The immunoprecipitate was analyzed by immunoblotting with a goat anti-mouse secondary antibody conjugated to HRP alone (lane 1) or anti-Caspase 7 (2µg/ml) followed by a goat anti-mouse secondary antibody conjugated to HRP (lane 3). Bands were visualized with a chemiluminescence detection system.

Immunoblot Analysis

Lanes 2 and 4: Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with a goat anti-mouse secondary antibody conjugated to HRP alone (lane 2) or anti-Caspase 7 (2 µg/ml) followed by a goat anti-mouse secondary antibody conjugated to HRP (lane 4). Bands were visualized using a chemiluminescence detection system. Arrow indicates Caspase 7 (~37kDa).

Application References:

1. Kirch, D. G., *et al.*, *J. Biol. Chem.* **274**: 21155-21161, 1999

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 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 30 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2.0mg/ml of anti-Caspase 7**, 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).

Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4mg of anti-Caspase 7** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l packed beads) of washed Protein G agarose bead slurry, Catalog # 16-266.
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 60 μ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.