



cell signaling solutions

Certificate of Analysis

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Anti-Caspase 1/ICE

(rabbit polyclonal IgG)

Catalog # 06-503

Lot # 26416

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acid residues 129-149 of human Caspase 1 (PEHKTSDSTFLVFM SHGIREG).

Specificity: Recognizes the p45 proenzyme, Mr 45kDa, and the p20 subunit, Mr 20kDa, of reduced human Caspase 1 isoforms.

Species Cross Reactivity: Human, mouse and rat.

Formulation: 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl containing 0.05% sodium azide. Frozen solution.

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

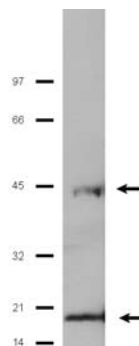
FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

Immunoblot Analysis: 0.5-2µg/ml of this lot of antibody detected the p45 proenzyme form of Caspase 1 in 20µg of cell lysate from human HL-60. The immuno-reactivity can be inhibited by the immunizing peptide.

Immunoprecipitation: 5µg of a previous lot of this antibody immunoprecipitated p45 proenzyme form of Caspase 1 from 500µg of human HL-60 cell lysates.

Immunocytochemistry: 10µg/ml of a previous lot of this antibody gave positive immunostaining of HL60 cells.



Immunoblot Analysis:

Representative blot from a previous lot. HL-60 cell lysate was resolved by electrophoresis, transferred to nitro-cellulose and probed with anti-Caspase 1/ICE (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicates caspase 1 (20kDa) and pro-caspase 1 (45kDa).

Application References:

1. Martini, J., *et al.*, *Mol. Endocrinol.* **14**: 1536-1549, 2000.
2. Takeuchi, R., *et al.*, *J. Virol.* **72**: 4498-4502, 1998.

General References:

3. Cohen, G.M., *Biochem. J.* **326**: 1-16, 1997.
4. Molineux, *et al.*, *PNAS* **90**: 1809, 1993.

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 EDTA; 1mM PMSF; 1 μ g/ml 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), (Catalog # 20-200), for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-Caspase 1/ICE**, 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 twice with water.
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. Before beginning the immunoprecipitation, dilute the cell lysate to roughly 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **5 μ g of anti-Caspase 1/ICE**, 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 or A agarose bead slurry.
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 and boil 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, or the agarose beads can then be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.

Immunocytochemistry

1. Plate approximately 200 μ l of cell suspension into each well of a slide. Incubate 24 hours. in a 37°C CO₂ incubator.
2. Wash the cells three times for 5 minutes with PBS. Do not shake cells.
3. Add fix (ice-cold ethanol/acetic acid [95:5]) for 1 minute at room temperature.
4. Wash the cells with PBS, twice, for 5 minutes. Do not shake.
5. Cover the cells with of 1% albumin in PBS and incubate for 60 minutes at room temperature.
6. Wash the cells two times with PBS, for 5 minutes per wash.
7. Cover the cells with **10 μ g/ml anti-Caspase 1/ICE**, in 1% albumin in PBS and incubate for 2 hours at room temperature or overnight at 4°C in a humidified chamber.
8. Wash the cells twice with PBS, for 15 minutes.
9. Incubate the cells in the dark, with a **1:200 dilution of goat anti-rabbit IgG** fluorescein conjugated secondary antibody in PBS for 90 minutes at room temperature.
10. Wash the cells three times with PBS, for 5 minutes.
11. Examine the cells under a fluorescent microscope.