



cell signaling solutions

Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstate.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstate.com

Anti-Caspase 1/ICE

(rabbit polyclonal IgG)

Catalog # 06-503

Lot # 20910

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acid residues 129-152 of human Caspase 1 (PEHKTSDSTFLVFMHSHGIREG).

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.

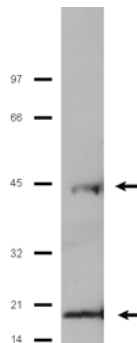
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing and Research Applications

Western 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).

Western 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 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 20-30 minutes at 20-25°C 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 IgG** linked to horseradish peroxidase, 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).

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 of washed Protein G or A agarose bead slurry (50 μ l packed beads).
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 50 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 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 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.