

**Anti-Human ICE (p20 subunit)**

**Catalog # 06-503**

**Lot # 15672**

**Background:** Interleukin-1 $\beta$  converting enzyme (ICE) is a cytoplasmic cysteine protease that cleaves the inactive form of interleukin1- $\beta$  (IL-1 $\beta$ ) to generate the active form of this proinflammatory cytokine (1,2). ICE is expressed in many tissues as a proenzyme of 45kDa, which is activated by proteolytic cleavage (3,4). Active ICE is composed of two subunits, known as p10 (10kDa) and p20 (20kDa), respectively. Analysis of ICE protein crystals indicate that these subunits associate as a tetramer with the stoichiometry (p20)<sub>2</sub>/(p10)<sub>2</sub> (5,6). ICE has unusual substrate specificity as it preferentially cleaves its substrates after Asp residues (7,8). This protease belongs to an emerging family of cysteine proteases that are important in regulating programmed cell death or apoptosis (9,10). ICE is functionally homologous to the protein encoded by the *C. elegans* death gene *ced-3* (11) and overexpression of ICE in fibroblasts induced apoptosis (9,11). In addition, overexpression of Crm A, a poxviral protein that specifically inhibits ICE (12), prevented apoptosis in fibroblasts and protected ganglion cells from apoptosis following NGF withdrawal (11,13).

**Immunogen:** Synthetic peptide (PEHKTS DSTFLVFM SHGIREG) corresponding to amino acid residues 129-152 of human ICE, conjugated to KLH.

**Specificity:** Recognizes p20 (20kDa) subunit of reduced human ICE isoforms and the p45 (45kDa) proenzyme.

**Species Cross Reactivity:** Mouse and rat.

**Quantity and Formulation:** 200 $\mu$ g protein A-purified rabbit IgG in 200 $\mu$ l of 0.1M Tris-glycine, pH 7.4 with 0.05% sodium azide.

**Physical Form:** Frozen liquid.

**Storage and Shelf Life:** 2 years at -20 $^{\circ}$ C from time of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot to avoid repeated freezing and thawing.

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

**Background References:**

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4. Thornberry, N.A., *et. al.*, Nature **356**: 768-774, (1992).
5. Wilson, K.P., *et. al.*, Nature **370**: 270-275, (1994).
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7. Sealath, P.R., *et. al.*, J. Biol. Chem. **265**: 14526-14528, (1990).
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11. Miura, M., *et. al.*, Cell **75**: 653-660, (1993).
12. Ray, C., *et. al.*, Cell **69**: 597-604, (1992).
13. Gagliardini, V., *et. al.*, Science **263**: 826-828, (1994)

**Additional References:**

- Molineaux, *et. al.*, PNAS **90**: 1809-1813, (1993).  
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### Quality Control Testing and Research Applications

Western Immunoblot Analysis: Used at 0.5-2 $\mu$ g/ml, this lot of antibody detected the p45 proenzyme form of ICE in 20 $\mu$ g of cell lysate from human HL-60 cells and mouse ABE 8 1/2 cells, respectively. The immunoreactivity can be inhibited by the immunizing peptide.

Immunoprecipitation: 5 $\mu$ g of this lot of antibody immunoprecipitated p45 proenzyme form of ICE from 500 $\mu$ g of human HL-60 cell lysates.

Immunocytochemistry: At 10 $\mu$ g/ml, this lot of antibody gave positive immunostaining of HL60 cells.

### 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 $\mu$ g/ml 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 PBS containing 3% nonfat dry milk (MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2ng/ml of a-Human ICE (p20 subunit)**, 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, 1:3000 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  $1\mu\text{g}/\mu\text{l}$  total cell protein in a microcentrifuge tube with PBS.
2. Add **5ng of a-Human ICE (p20 subunit)**, to 500 $\mu\text{g}$ -1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu\text{l}$  of washed Protein G or A agarose bead slurry (50 $\mu\text{l}$  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 $\mu\text{l}$  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 $\mu\text{l}$  of cell suspension into each well of a slide. Incubate 24 hr. in a 37°C CO<sub>2</sub> 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 15 minutes. Do not shake.
5. Cover the cells with of 8% albumin in PBS and incubate for 30 minutes at room temperature.
6. Wash the cells two times with PBS, for 15 minutes per wash.
7. Cover the cells with **10ng/ml a-Human ICE (p20 subunit)**, in 1% albumin in PBS and incubate overnight at 4°C in a humidified chamber.
8. Wash the cells twice with PBS, for 15 minutes.
9. Incubate the cells with a **1:100 dilution of goat anti-rabbit IgG** fluorescein conjugated secondary antibody in PBS for 1 hour at room temperature.
10. Wash the cells three times with PBS, for 5 minutes.
11. Examine the cells under a fluorescent microscope.