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Certificate of Analysis

Anti-Caspase-8 (N-terminus), clone E6, rabbit monoclonal
(Rabbit monoclonal IgG)
Catalog # 04-574
Lot #

Immunogen: A synthetic peptide corresponding to N-terminal residues of human Caspase-8. The antibody should recognize all splice isoforms of Caspase-8.

Specificity: Recognizes human Caspase-8 (N-Term).

Molecular Weight: 55 kDa.

UniProt ID: Q14790

Species Cross-reactivity: Reacts with human. Not believed to cross-react with mouse or rat.

Formulation: 100 µL of rabbit monoclonal IgG in 50mM Tris-Glycine (pH 7.4), 0.15 M NaCl, 0.01% sodium azide, 0.05% BSA and 40% glycerol.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/ thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

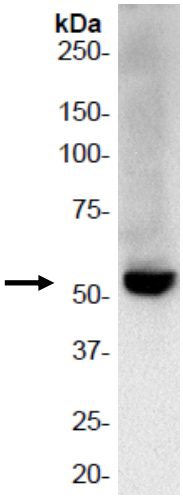
Applications:

Western blot: A 1:500-1:1000 dilution

Immunoprecipitation: 1:50 dilution

Immunohistochemistry: 1:100 dilution

Flow cytometry: 1:50 dilution



Quality Control Testing:

Western blot Analysis:
Lysate from Jurkat cells was resolved by electrophoresis, transferred to PVDF and probed with anti-caspase-8 (1:1,000). Proteins were visualized using goat anti-rabbit secondary antibody conjugated to HRP and chemiluminescence detection. Arrow indicates caspase-8, (55 kDa).

General References:

1. Medema, J.P. et al. (1997). FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J.* **16(10)**: 2794–804.
2. Muzio, M. et al. (1996). FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* **85**: 817–827.
3. Boldin, M.P. et al. (1996). Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. *Cell* **85**: 803–815.
4. Srinivasula, S.M. et al. (1996). Molecular ordering of the Fas-apoptotic pathway: the Fas/APO-1 protease Mch5 is a CrmA-inhibitable protease that activates multiple Ced-3/ICE-like cysteine proteases. *Proc. Natl. Acad. Sci. U S A* **93(25):** 14486–91.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate samples (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 μg/mLl each aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4, 1 mM NaF) and transfer the proteins to PVDF. Wash the blotted PVDF twice with TBST.
2. Block the blotted PVDF in freshly prepared 5% BSA in TBS with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with 1:1,000 dilution of **anti-caspase-8** diluted in freshly prepared 5% BSA in TBST for 2 hours at room temperature or overnight with agitation at 4°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST/5% BSA for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use detection method of choice (enhanced chemiluminescence was used).

Rabbit Monoclonals Produced Using Technology from Epitomics, Inc. Under Patent No. 5,675,063