

Certificate of Analysis

Anti-FADD, clone 1F7

(mouse monoclonal IgG₁)

Catalog # 05-486

Lot # 32331

Immunogen: GST-tagged full-length fusion protein corresponding to human FADD. Clone 1F7.

Specificity: Recognizes FADD, Mr 28kDa.

Species Cross-reactivity: Human and mouse.

Formulation: 100µg of protein A purified mouse IgG₁ in 100µl of PBS, pH 7.2 and 50% glycerol.

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

Handling Recommendations: Upon receipt, 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.

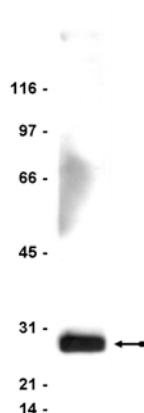
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected FADD in RIPA lysates from Jurkat cells.

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

Immunoprecipitation: This antibody has been demonstrated by an independent laboratory to immunoprecipitate FADD.³



Immunoblot Analysis

Representative blot from a previous lot. Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-FADD (1µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FADD (~28kDa).

Application Reference:

1. McDonald III, E., *et al.*, *J. Biol. Chem.* **276**: 14939-14945, 2001.
2. Curtin, J. F. and Cotter, T. G., *J. Biol. Chem.* **279**: 17090-100, 2004.
3. Xerri, L., *et al.*, *Brit. J. Haema.* **106**: 652-62, 1999.

General Reference:

4. Chinnaiyan, A.M., *et al.*, *Cell* **81**: 505-512, 1995.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 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 (Catalog # 20-200), (PBS-MLK) for 20-60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-FADD, clone 1F7**, 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, Catalog # 12-349, 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 15 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).