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## Certificate of Analysis

**Anti-FADD, clone 1F7**  
(mouse monoclonal IgG<sub>1</sub>)  
Catalog # 05-486  
Lot # 21758

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

**Specificity:** Recognizes FADD, Mr 28kDa.

**Species Cross-reactivity:** Human and mouse.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**Formulation:** 100mg of protein A purified mouse IgG<sub>1</sub> in 100ml of PBS, pH 7.2 and 50% glycerol.

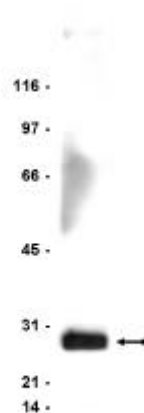
**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

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### Quality Control Testing

**Western 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 2.5ml of 2-mercaptoethanol per 100ml of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.



### 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).

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### General Reference:

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

### Western 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 EGTA; 1mM PMSF; 1µg/ml each 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 (PBS-MLK) for 60 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2mg/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, 1:2000 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).