
Certificate of Analysis

Anti-FADD, clone 1F7
(mouse monoclonal IgG₁)
Catalog # 05-486
Lot # 20931

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

Specificity: Recognizes FADD, 28kDa.

Species Cross-reactivity: Human and mouse.

Storage and Stability: Lyophilized: Stable for 2 years at 4°C from date of shipment. Rehydrated: Stable for 1 month at 4 °C, 6 months at -20 °C. Aliquot to avoid repeated freezing and thawing.

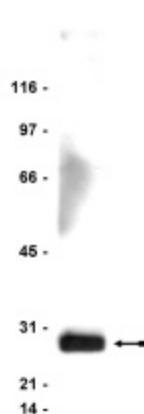
Formulation: **100mg** of protein A purified mouse IgG₁ lyophilized from **100ml** of PBS, pH 7.2 with 1% sucrose. Rehydrate the product in **100ml** of distilled water.

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

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 2.5µl 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.



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).

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; 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 (PBS-MLK) for 60 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2mg/ml of anti-FADD**, 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, 1:3000 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 15 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).