

Anti-FADD

(mouse monoclonal IgG₁)

Catalog # 05-486

Lot # 17128

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

Hybridoma: Clone IF7 established by fusion of mouse myeloma P3U1 with Balb/c splenocytes.

Specificity: Specific for 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.

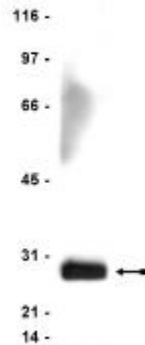
Formulation: 100ng of protein A purified IgG₁ lyophilized from 100ml of PBS, pH 7.2 with 1% sucrose. Rehydrate the product in 100ml of distilled water.

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

Quality Control Testing

Immunoblot Analysis: 1-2µg/ml of this lot detected FADD in RIPA lysates from Jurkat cells.

Included Positive Antigen Control: Catalog # 12-303, Jurkat lysate. Use 20µg per lane for minigels.



Immunoblot Analysis

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).

Background: FADD was originally isolated as a protein that bound to the cytoplasmic domain of Fas in the yeast two-hybrid system. Sequence analysis revealed a region homologous to the death domain of Fas and TNFR-1. Subsequent biochemical studies have shown that FADD associates with FAS through interaction of the death domains. When over expressed in several cell lines, FADD induces apoptosis, which can be blocked by CrmA, an inhibitor of interleukin-1-beta-converting enzyme. This evidence suggests that FADD plays a role in Fas-mediated apoptosis.

General References:

Dixit, *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 EGTA; 1mM PMSF; 1µg/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 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1-2µg/ml of α-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 α-mouse** HRP conjugated IgG, 1:1,000 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).