



cell signaling solutions

Certificate of Analysis

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Anti-Fas, clone 7C10

(rat monoclonal IgG_{2a})

Catalog # 05-351

Lot # 24194

Immunogen: recombinant protein containing the cytoplasmic domain of mouse Fas. Clone 7C10

Specificity: Recognizes Fas, Mr 45kDa.

Species Cross-reactivity: Mouse and bovine

Formulation: 200µg of protein G-purified rat IgG_{2a} in 200µl of 0.1 M Tris-glycine, 0.15 M NaCl, pH 7.4 with 0.05% sodium azide. Frozen solution

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

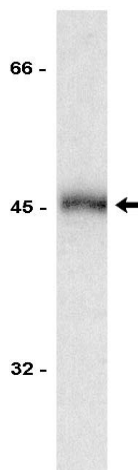
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing and Research Applications

Immunoblot Analysis: 0.5-2µg/ml of this lot detected Fas in RIPA lysates of mouse 3T3/A31 fibroblasts. A previous lot detected Fas in membrane preparations of bovine brain.

Included Positive Antigen Control: Catalog #12-305, 3T3/A31 cell lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

Immunoprecipitation: 4µg of a previous lot immunoprecipitated Fas from mouse 3T3/A31 cells. RIP, a death domain containing protein, was co-precipitated with Fas (1).



Immunoblot Analysis

Representative blot lot from a previous lot. 3T3/A31 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Fas (0.5µg/ml). Proteins were visualized using a goat anti rat secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Fas (~45kDa).

Application References:

1. Stanger, B.Z., et al., Cell 81:513-523, 1995

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml 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 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose in **0.5-2 μ g/ml of anti-Fas** 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-rat HRP conjugated IgG, 1:1000 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 5-10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Add 500 μ g-1mg of cell lysate at a concentration of roughly 1 μ g/ μ l total cell protein to a microcentrifuge tube.
2. Add **4 μ g of anti-Fas** to the tube.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 60 μ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.