

Anti-Mouse Fas
(rat IgG2a, clone 7C10)

Catalog # 05-351

Lot # 14886

Background: Fas is an apoptosis-signaling receptor molecule found on the surface of a number of cell types and belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family.

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

Specificity: Recognizes Fas (about 45 kDa)

Species Cross-reactivity: Bovine

Quantity and Formulation: **200 mg** of protein G-purified rat IgG in **200 ml** of 0.1 M Tris-glycine, 0.15 M NaCl, pH 7.4 with 0.05% NaN₃.

Physical Form: Frozen liquid

Storage and Stability: 2 years at -20 C. 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.

References:

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

FOR RESEARCH USE ONLY.
NOT FOR USE IN HUMANS.

Quality Control Testing and Research Applications

Western Immunoblot Analysis: This lot was tested by using 0.5 and 2.0 µg/ml of the antibody to detect Fas (~45 kDa) in membrane preparations of mouse 3T3 cells and bovine brain, respectively.

Immunoprecipitation: This lot was tested by using 4 µg of the antibody to immunoprecipitate Fas from 3T3 cells. RIP, a death domain containing protein, was co-precipitated with Fas (1).

Western 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; 1µg/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 (MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose in **0.5-2.0mg/ml of a-Mouse 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 IgG linked to horseradish peroxidase, 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 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 500µg-1mg of cell lysate at a concentration of roughly 1µg/µl total cell protein to a microcentrifuge tube.
2. Add **4ng of a-Mouse Fas** to the tube.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein G agarose bead slurry (50µl packed beads).
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 50µl 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. The beads are collected by a microcentrifuge pulse and SDS-PAGE and subsequent Immunoblot analysis can be performed on a sample of the supernatant.