

**Anti-Human Fas (activating)**  
**(mouse immunoaffinity purified IgM)**  
**Catalog # 05-201**  
**Lot # 18787**

**Immunogen:** FS-7 (human diploid fibroblast cell line).

**Antibody Class:** Mouse IgM. Produced by NS-1 myeloma x Balb/c splenocyte hybridoma, clone CH-11, propagated as ascites.

**Specificity:** This antibody recognizes the human cell surface antigen Fas (~43kDa) expressed in various human cells, including myeloid cells, T lympho-blastoid cells, and diploid fibroblasts.

**Biological Activity:** The antibody demonstrates cytolytic activity on human cells that express Fas. Murine WR19L cells and L929 cells transfected with cDNA encoding human Fas undergo apoptosis in response to this antibody.

**Cross-reactivity:** This antibody does not recognize TNF, and does not cross-react with mouse Fas. Fas Ligand (Catalog # 01-193) will induce apoptosis in human, mouse and rat systems.

**Formulation:** **50mg** of mouse immunoaffinity purified IgM in **100ml** of PBS containing 50% glycerol. Liquid at -20°C.

**Storage and Stability:** Stable for 1 year at -20°C from date of shipment. For maximum recovery of the product, centrifuge the original vial prior to removing the cap.

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

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**Quality Control Testing**

Apoptosis: >30ng/ml of this lot maximally induced apoptosis of human Jurkat cells with 70-80% mortality after 24 hours of treatment. This lot has an ID<sub>50</sub> of 1-4ng/ml in apoptosis assays.

Immunoblot Analysis: 0.5-2µg/ml of this lot detected Fas in a HeLa cell extract.

Immunocytochemistry: 10µg/ml of this lot detected Fas on HeLa cells fixed with 4% para-formaldehyde/2% acetic acid.

**Additional Research Applications**

Flow cytometry: Use 20µg/ml.<sup>1,2</sup>

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**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.

**References:**

1. Yonehara, S., *et al.*, J. Exp. Med. **169**: 1747-1756, 1989.
2. Kobayashi, N., *et al.*, Proc. Natl. Acad. Sci. USA **87**: 9620-9624, 1990.
3. Itoh, N., *et al.*, Cell **66**: 233-243, 1991.

### Apoptosis Assay Protocol

#### A. Day One - Treating Cells

1. Fill a 96 well microtiter plate with 100 $\mu$ l of cell growth media (RPMI, 2% FBS).
2. Add 100 $\mu$ l of **a-Fas** (at 4x final concentration) in duplicate to row 1.
3. Titrates 100 $\mu$ l from row 1 serially across the plate.
4. Add 100 $\mu$ l of Jurkat cells to each well at a density of  $10^5$  cells/well.
5. Incubate for 20-24 hours at 37°C, 5% CO<sub>2</sub>.

#### B. Day Two - Cell Viability Check - MTT

1. Add 10 $\mu$ l of 5mg/ml MTT in PBS to all wells.
2. Incubate for 2 hours at 37°C, 5% CO<sub>2</sub>.
3. Spin the plate at 2,500 rpm for 5 minutes to pellet the cells.
4. Gently aspirate off the culture media.
5. Add 100 $\mu$ l of acidic isopropanol (0.1N HCl) to each well.
6. Mix until the MTT crystals dissolve, approximately 5 minutes at room temperature.
7. Read absorbance at 570nm.
8. Compare absorbance of wells treated with **a-Fas** to wells not treated with **a-Fas**, an apoptosis inducing agent.

### 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 $\mu$ g/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 0.05% Tween 20 and 3% nonfat dry milk (PBST-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2ng/ml of a-Fas**, diluted in freshly prepared PBST-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 a-mouse IgM** HRP conjugated IgG, 1:2000 dilution was used) in PBST-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 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Wash the nitrocellulose for 2 hours in water.
10. Use detection method of choice (enhanced chemiluminescence was used).

### Immunocytochemistry Protocol

1. Plate approximately 200 $\mu$ l of cell suspension into each well of a slide. Incubate 24 hours at 37°C, 5% CO<sub>2</sub>.
2. Wash the cells three times for 5 minutes with PBS. Shake cells slowly.
3. Fix the cells with ice-cold 4% paraformaldehyde/2% acetic acid in PBS for 10 minutes at 4°C.
4. Wash the cells with 400 $\mu$ l PBS, twice, for 5 minutes. Shake slowly.
5. Cover the cells with 8% BSA in PBS and incubate for 1 hour at room temperature.
6. Cover the cells with **5-10ng/ml a-Human Fas** in 1% BSA in PBS and incubate overnight at 4°C. Also run a negative control antibody to check for non-specific staining.
7. Wash the cells twice with PBS, for 5 minutes.
8. Incubate the cells with a **1:200 dilution of goat anti-mouse IgM** fluorescein conjugated secondary antibody in PBS for 1.5 hours at room temperature.
9. Wash the cells three times with PBS, for 5 minutes.
10. Examine the cells under a fluorescent microscope.