

## Anti-Human Fas (Activates)

(mouse monoclonal IgM)

Catalog # 05-201

Lot # 17669

**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. Cold solution.

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

**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: 5-10µg/ml of this lot detected Fas on HeLa cells fixed with 4% para-formaldehyde/2% acetic acid.

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

### Additional Research Applications

Apoptosis: Use 50-500ng/ml to induce apoptosis of cells.<sup>1-3</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 100µl of cell growth media (RPMI, 2% FBS).
2. Add 100µl of **a-Human Fas** (at 4x final concentration) in duplicate to row 1.
3. Titrate 100µl from row 1 serially across the plate.
4. Add 100µl 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 10µl 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 100µl 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-Human Fas** to wells not treated with **a-Human 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; 1µg/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 (TPBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2ng/ml of a-Human Fas**, diluted in freshly prepared TPBS-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 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 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

### Immunocytochemistry Protocol

1. Plate approximately 200µl 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 400µl PBS, twice, for 15 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 15 minutes.
8. Incubate the cells with a **1:100 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 15 minutes.
10. Examine the cells under a fluorescent microscope.