

**Certificate of Analysis**

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**Anti-Fas (human, activating), clone CH11  
(mouse immunoaffinity purified IgM)****Catalog # 05-201****Lot # JBC1394138**

**Immunogen:** FS-7 (human diploid fibroblast cell line). Clone CH-11.

**Specificity:** This antibody recognizes the human cell surface antigen Fas, Mr 43 kDa expressed in various human cells, including myeloid cells, T lymphoblastoid 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 will induce apoptosis in human, mouse and rat systems.

**Formulation:** 50 µg of mouse immunoaffinity purified IgM in 100 µL of PBS, pH 7.2, 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:** 15-20 ng/mL of this lot maximally induced apoptosis of human Jurkat cells with 83% mortality after 24 hours of treatment.

**Immunoblot Analysis:** 0.5-2 µg/mL of this lot detected Fas in a HeLa cell lysate.

**Immunocytochemistry:** 5-10 µg/mL of a previous lot detected Fas on HeLa cells fixed with 4% formalin/2% acetic acid.

**Additional Research Applications**

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

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**Application 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.
4. Bennett, M., *et al.*, *Science* **282**: 290-293, 1998.
5. Gutierrez-Steil, C., *et al.*, *J. Clin. Invest.* **101**: 33-39, 1998.

### 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 **anti-Fas (human, activating), clone CH11** (at 4x final concentration) in duplicate to row 1.
3. Titrate 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>. Determine cell viability by using ATP endpoint assay or MTT, see B or C below.

#### B. Day Two – Cell Viability Check – ATP

Assay for cell number using ATP endpoint assay per vendor's instructions. Read ATP levels and compare absorbance of cells that were allowed to grow maximally to cells that were induced to undergo apoptosis in the presence or absence of **anti-Fas (human, activating), clone CH11**, an apoptosis inducing agent.

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

1. Add 10  $\mu$ L of 5 mg/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 (0.1 N HCl) to each well.
6. Mix until the MTT crystals dissolve, approximately 5 minutes at room temperature.
7. Read absorbance at 570 nm.
8. Compare absorbance of wells treated with **anti-Fas (human, activating), clone CH11** to wells not treated with **anti-Fas (human, activating), clone CH11**, an apoptosis inducing agent.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1  $\mu$ g/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM 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) and 0.05% Tween 20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2  $\mu$ g/mL of anti-Fas (human, activating), clone CH11**, 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 anti-mouse IgM** HRP conjugated IgG, Catalog # 12-489, 1:5,000 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 1 hour 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% formalin/2% acetic acid in PBS for 12 minutes at 4°C.
4. Wash the cells with 400  $\mu$ L PBS, twice, for 15 minutes with no agitation.
5. Cover the cells with 400  $\mu$ L 1% BSA in PBS and incubate for 1 hour at room temperature.
6. Wash the cells with 400  $\mu$ L PBS, twice, 15 minutes with no agitation.
7. Cover the cells with **5-10  $\mu$ g/mL anti-Fas (human, activating), clone CH11** in 1% BSA in PBS and incubate overnight at 4°C or 2 hours at room temperature. Also run a negative control antibody to check for non-specific staining.
8. Wash the cells twice with PBS, for 5 minutes.
9. Incubate the cells in the dark, with a **1:200 dilution of goat anti-mouse IgM** fluorescein conjugated secondary antibody in 1% BSA in PBS for 1.5 hours at room temperature.
10. Wash the cells three times with PBS, for 5 minutes.
11. Examine the cells under a fluorescent microscope.