upstate
cell signaling solutions

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Certificate of Analysis

Anti-Fas (human, activating), clone CH11

(mouse immunoaffinity purified IgM)
Catalog # 05-201
Lot # 23654

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

Specificity: This antibody recognizes the human cell surface antigen Fas, Mr ~43kDa 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 (Catalog # 01-193) will induce apoptosis in human, mouse and rat systems.

Formulation: **50μg** of mouse immunoaffinity purified IgM in **100μl** 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

Quality Control Testing

Apoptosis: 15-20ng/ml of this lot maximally induced apoptosis of human Jurkat cells with 80-90% mortality after 24 hours of treatment. This lot has an ID_{50} of 1.95ng/ml in apoptosis assays.

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

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

Additional Research Applications

Flow cytometry: Use 20μg/ml.[1, 2]

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 100µl of cell growth media (RPMI, 2% FBS).
2. Add 100µl of **anti-Fas (human, activating), clone CH11** (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 10^5cells/well.
5. Incubate for 20-24 hours at 37°C, 5% CO_2. 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 10µl of 5mg/ml MTT in PBS to all wells.
2. Incubate for 2 hours at 37°C, 5% CO_2.
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 **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: 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_3VO_4; 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) and 0.05% Tween 20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/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, 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 200µl of cell suspension into each well of a slide. Incubate 24 hours at 37°C, 5% CO_2.
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 400µl PBS, twice, for 15 minutes with no agitation.
5. Cover the cells with 400µl 1% BSA in PBS and incubate for 1 hour at room temperature.
6. Wash the cells with 400µl PBS, twice, 15 minutes with no agitation.
7. Cover the cells with **5-10µg/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.