



Certificate of Analysis

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Anti-Human Fas (neutralizes)

(mouse monoclonal IgG₁, clone ZB4)

Catalog # 05-338

Lot # 18388

Immunogen: Recombinant human Fas.

Antibody Class: Mouse IgG₁. Produced by NS-1 myeloma x Balb/c splenocyte hybridoma, propagated as ascites. Clone ZB4.

Specificity: This antibody recognizes the human cell surface antigen Fas expressed in various human cells, including myeloid cells, T lympho-blastoid cells and diploid fibroblasts. It does not induce apoptosis in cell culture.

Species Cross-reactivity: This antibody does not recognize TNF and does not cross-react with mouse Fas.

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

Formulation: 100mg of ammonium sulfate and protein A purified mouse IgG₁, lyophilized from 100µl PBS containing 10mg/ml sucrose. Lyophilized powder. Reconstitute in 100µl distilled water.

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

Quality Control Testing

Neutralization: This lot of antibody, at a range of 62.5 to 250ng/ml, neutralized over 80% of the apoptosis induced by anti-Human Fas, clone CH-11 (Catalog # 05-201, 50ng/ml) treatment of human Jurkat cells.

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.

Apoptosis Neutralization Assay Protocol

A. Day One – Setting up plates and treating cells

1. Add 50µl of cell growth medium (RPMI, 2% FBS) to the wells of a 96 well microtiter plate.
2. Add 50µl of **α-Human Fas, clone ZB4** (at 4x final concentration) in duplicate to row 1.
3. Titrate 50µl from row 1 serially across the plate.
4. Add 50µl of Jurkat cells to each well at a density of 10^5 cells/well.
5. Incubate the plate at 37°C, 5% CO₂ for 1 hour to allow antibody/cell interaction.
6. Add 100µl of apoptosis inducing α-Human Fas, clone CH-11 (Catalog # 05-201), diluted in cell growth medium to 50ng/ml final concentration.
7. Incubate for 20-24 hours at 37°C, 5% CO₂.

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% CO₂.
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. Fill 4 or more wells with 100µl of acidic isopropanol and use to zero the microtiter plate reader
8. Read at 570nm.
9. Compare absorbance of cells that were allowed to grow maximally to cells that were induced to undergo apoptosis in the presence or absence of the neutralizing antibody α-Human Fas, clone ZB4.