

## Certificate of Analysis

**Anti-Fas (human, neutralizing), clone ZB4**  
(mouse monoclonal IgG<sub>1</sub>)  
Catalog # 05-338  
Lot # 0701049387

**Immunogen:** Recombinant human Fas. 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 mouse Fas.

**Formulation:** 100 µg of ammonium sulfate and protein A purified mouse IgG<sub>1</sub>, in 100 µL PBS containing 50% glycerol. Store at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

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

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### Quality Control Testing

**Neutralization:** 500 ng/mL of this lot neutralized over 50% of the apoptosis induced by anti-Fas (human, activating), clone CH11 (Catalog # 05-201, 50 ng/mL) treatment of human Jurkat cells. Cell viability was assessed using an ATP endpoint assay (ATPLite™-M, Packard Instruments).

**Flow Cytometry:** Use 5-20 µg/mL.

**Immunoblotting:** Use 5 µg/mL.

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### References:

1. Zhang, B., *et al* (2003). Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross-talk between caspase 8, reactive oxygen species, and MAPK/ERK activation. *J Biol Chem* **278**: 28443-54.
2. Lacana, E. and D'Adamio, L. (1999). Regulation of Fas ligand expression and cell death by apoptosis-linked gene 4. *Nat Med* **5**: 542-7.
3. Boirivant, M., *et al* (1996). Stimulated human lamina propria T cells manifest enhanced Fas-mediated apoptosis. *J Clin Invest* **98**: 2616-22.
4. Yonehara, S., *et al* (1994). Involvement of apoptosis antigen Fas in clonal deletion of human thymocytes. *Int Immunol* **6**: 1849-56.

### Example Apoptosis Neutralization Assay Protocol

#### A. Day One – Setting up plates and treating cells

1. Add 50  $\mu$ L of cell growth medium (RPMI, 2% FBS) to the wells of a 96 well microtiter plate. **NOTE:** Use black microtiter plate if performing ATP endpoint assay.
2. Add **50  $\mu$ L of anti-Fas, (human, neutralizing)** (at 4x final concentration) in duplicate to row 1.
3. Titrate 50  $\mu$ L from row 1 serially across the plate.
4. Add 50  $\mu$ L of Jurkat cells to each well at a density of  $10^5$  cells/well.
5. Incubate the plate at 37°C, 5% CO<sub>2</sub> for 1 hour to allow antibody/cell interaction.
6. Add 100  $\mu$ L of apoptosis inducing anti-Human Fas, clone CH-11 (Catalog # 05-201), diluted in cell growth medium to 50 ng/mL final concentration.
7. 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 the neutralizing antibody anti-Fas (human, neutralizing).

#### 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 (0.1N HCl) to each well.
6. Mix until the MTT crystals dissolve, approximately 5 minutes at room temperature.
7. Fill 4 or more wells with 100  $\mu$ L of acidic isopropanol and use to zero the microtiter plate reader
8. Read at 570 nm.

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 anti-Fas (human, neutralizing).