



## Monoclonal Anti-feline Fas/TNFRSF6 Antibody

### ORDERING INFORMATION

**Catalog Number:** MAB2267

**Clone:** 431014

**Lot Number:** ZRV01

**Size:** 100 µg

**Formulation:** 0.2 µm filtered solution in PBS with 5% trehalose

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** feline Fas  
extracellular domain

**Immunogen:** NS0-derived rfeFas  
extracellular domain

**Ig class:** mouse IgG<sub>1</sub>

**Recommended Application:**  
Flow cytometry

**Other Applications:**  
Western blot  
Direct ELISA

### Background

Fas, also known as CD95 and TNFRSF6, is a 45 kDa type I transmembrane glycoprotein. The ECD contains two cysteine-rich domains, and the cytoplasmic tail contains one pro-apoptotic death domain. Alternate splicing generates five additional isoforms of feline Fas, all of which lack the transmembrane segment. Fas binding to Fas Ligand triggers apoptosis through both the MEK cascade and FADD/caspase-8 pathway. Within the ECD feline Fas shares 68%, 65%, 53%, and 58% aa identity with human, mouse, porcine, and rat Fas, respectively.

### Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant feline Fas (rfeFas; aa 25 - 172; Accession # NP\_001009314) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

### Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

### Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

### Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

### Specificity

This antibody detects rfeFas in direct ELISAs and Western blots. In these applications, this antibody shows no cross-reactivity with rhFas, rmFas, or rrFas.

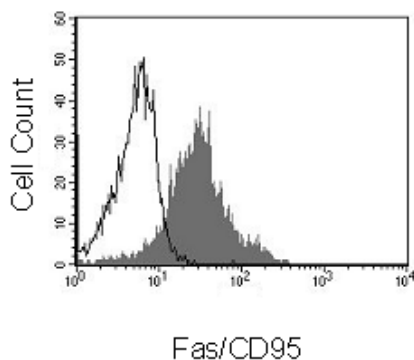
### Applications

**Flow cytometry** - This antibody was validated for flow cytometry using PMA plus Ca<sup>2+</sup> ionomycin-activated feline PBMC. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 x 10<sup>5</sup> cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled monoclonal antibodies may be visualized by adding a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome.

**Western blot** - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect feline Fas. Using a colorimetric detection system, the detection limit for rfeFas is approximately 25 ng/lane under non-reducing conditions. Use of this antibody under reducing conditions is not recommended. Chemiluminescent detection with WesternGlo™ Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold. In this application, the use of feline Fas monoclonal antibody, R&D Systems Catalog # MAB22671, is recommended.

**Direct ELISA** - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect feline Fas. The detection limit for rfeFas is approximately 3 ng/well.

**Optimal dilutions should be determined by each laboratory for each application.**



Feline PBMC activated with PMA plus Ca<sup>2+</sup> ionomycin were stained with MAB2267 or isotype control (R&D Systems, Cat. # MAB002, open histogram) followed by PE-conjugated anti-mouse antibody (R&D Systems, Cat. # F0102B).

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