

Anti-FasL (MAb H11) (FITC)
ANTI-CD95L, ANTI-CD178, ANTI-TNFSF 6
(Anti-Mouse FasL A11 Monoclonal Antibody)

CATALOG NO.: XA-1001F

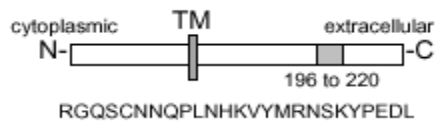
CLONE: H11

ISOTYPE: Rat IgG2a

CONJUGATE: FITC

BACKGROUND:

The immunogen is a synthetic peptide corresponding to amino acids 196-220 of mouse FasL (CD95L; APO-1L; CD178). The schematic structure of mouse FasL (peptide, aa. 196-220) is as follows:



SOURCE:

Anti-FasL (MAb H11) was raised against a synthetic peptide corresponding to amino acids 196-220 of mouse FasL. Anti-FasL (MAb H11) was purified to =95% in SDS-PAGE and is supplied at a concentration of 1mg/1ml. This product is FITC conjugated.

APPLICATION:

This FITC- labeled monoclonal antibody can be used for the detection of mouse FasL by flow cytometry and immunocytochemistry.

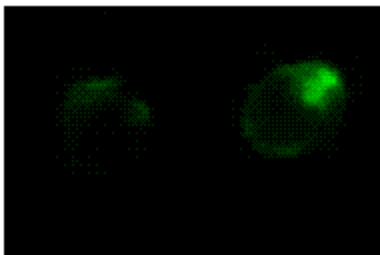


Figure: Detection of FasL in 293T cells transfected with a FasL expression vector.

Method: Transfected cells were plated onto polylysine treated glass slides, fixed and permeabilized in methanol at -20°C for 5 min, then in acetone at -20°C for 30 sec. After 3 washes in PBS, 0.1% BSA, were incubated for 1 hr at room temperature with 20 µg/ml of **biotinylated H11 antibody** in PBS, 0.1% BSA. After rinsing in PBS, FITC-

conjugated streptavidin was added for 30 min, slides washed again in PBS and visualized using a fluorescence microscope.

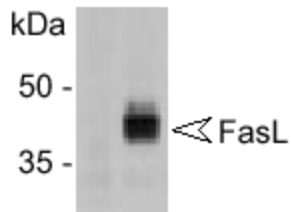


Figure: Detection of FasL in 293T cells transfected with a FasL expression plasmid (right panel). Mock-transfected cells (left panel).

Method: Cells extracts from (2×10^6) cells transfected with a FasL-expression plasmid were resolved by SDS-PAGE under reducing conditions, transferred to nitrocellulose and probed with the **monoclonal anti-FasL H11 antibody** at $2\mu\text{g/ml}$. Proteins were visualized using a peroxidase-conjugated antibody to rat IgG and a chemiluminescence detection svstem. Arrow indicates FasL.

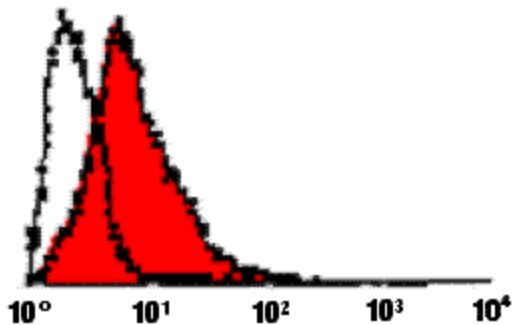


Figure: Flow cytometric profile of 293T cells transiently transfected with a FasL-expression plasmid (filled profile). The open profile corresponds to mock-transfected 293T cells.

Method: FasL-transfected 293T cells (5×10^5) were incubated on ice for 30 min in 50 μl FACS buffer (PBS, 5% Fetal calf serum, 0.02% azide) containing $1\mu\text{g}$ of **FITC-labeled H11 antibody**. After washing in FACS buffer, cells were analyzed by flow cytometry. Do not exclude pre-apoptotic cells during data acquisition (see T. Renno, M. Hahne, J. Tschopp and H. R. MacDonald, J. Exp. Med., **183**, 431 (1996)). **Note:** Activated primary T cells do not express high levels of surface FasL! **This product is for research use only.**



STORAGE:

This purified antibody is formulated as a liquid in PBS containing 0.02% sodium azide. This product should be shipped on blue ice, and long term storage is at 4°C. If stored at 4°C undiluted, this product will be stable for at least 6 months after receipt. Do not freeze/thaw.

REFERENCES:

1. Characterization of the non-functional Fas ligand of gld mice: M. Hahne, et al.; *Int. Immunol.* **7**, 1381 (1995).
2. CD4+ T cells reactivated with superantigen are both more sensitive to FasL-mediated killing and express a higher level of FasL: J.K. Wang, et al.; *Cell. Immunol.* **179**, 153 (1997).
3. Transgenic expression of CD95 ligand on thyroid follicular cells confers immune privilege upon thyroid allografts: L. Tourneur, et al.; *J. Immunol.* **167**, 1338 (2001).