

MONOCLONAL ANTIBODY

# Anti-CD8 (Mouse) mAb-FITC

Code No.	Clone	Subclass	Quantity
D271-4	KT15	Rat IgG2a $\lambda$	1 mL (100 tests)

**BACKGROUND:** The CD8 antigen is a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell-cell interactions within the immune system. The CD8 antigen, acting as a coreceptor, and the T cell receptor on the T lymphocyte recognize antigen displayed by an antigen presenting cell in the context of class I MHC molecules. The functional coreceptor is either a homodimer composed of two  $\alpha$  chains, or a heterodimer composed of one  $\alpha$  and one  $\beta$  chain. Both  $\alpha$  and  $\beta$  chains share significant homology to immunoglobulin variable light chains. CD8  $\alpha$  chains bind to class-I MHC molecules  $\alpha 3$  domains. CD8 identifies cytotoxic/suppressor T-cells that interact with MHC class I bearing targets. CD8 is thought to play a role in the process of T-cell mediated killing.

This monoclonal antibody KT15 reacts with a non-polymorphic epitope on the mouse CD8  $\alpha$  chain (mouse Ly2.1 and Ly2.2 cells).

**SOURCE:** This antibody was purified from mouse ascites fluid using protein G agarose. This hybridoma (clone KT15) was established by fusion of mouse myeloma cell NSO with SD rat splenocyte immunized with the T cell clone C6, H-2K<sup>k</sup> restricted H-Y antigen specific CTL.

**FORMULATION:** 100 tests in 1 mL volume of PBS containing 1% BSA and 0.1% ProClin 150.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with mouse CD8  $\alpha$  antigen on Flow cytometry.

**APPLICATION:**

Flow cytometry; 10  $\mu$ L (ready for use)

Detailed procedure is provided in the following **PROTOCOL**.

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cells	Not tested	Splenocyte	Not tested
Reactivity on FCM		+	

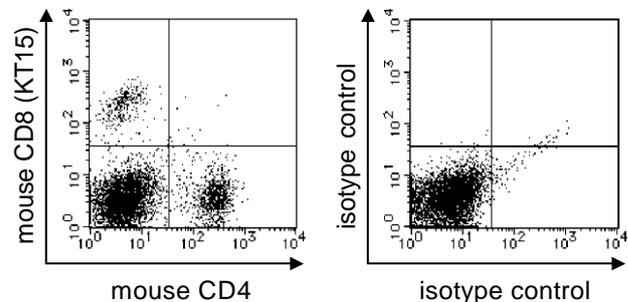
**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**REFERENCES:**

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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



**Flow cytometric analysis of mouse CD8 expression (left) and isotypic control (right) on mouse splenocytes. The staining intensity of D271-4 is shown in the vertical axis with mouse CD4 staining on the horizontal axis.**

**PROTOCOL:**

**Flow cytometric analysis for mouse splenocytes**

Single spleen cell suspensions are prepared from the spleens according to standard procedures. We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the hemolyzed splenocytes 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN<sub>3</sub>].  
\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 2) Resuspend the cells with washing buffer (2 x 10<sup>7</sup> cells/mL).
- 3) Add 50  $\mu$ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 20  $\mu$ L of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.

- 5) Add 10  $\mu$ L of Anti-CD8 (Mouse) mAb-FITC (clone KT15) as suggested in the **APPLICATION**, and add PE-conjugated anti-mouse CD4 antibody (clone GK1.5) to the each tube. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse splenocyte)

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