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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-CD8 (Mouse) mAb-FITC

Code No.	Clone	Subclass	Quantity
K0227-4	KT15	Rat IgG2a λ	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 α chains, or a heterodimer composed of one α and one β chain. Both α and β chains share significant homology to immunoglobulin variable light chains. CD8 α chains bind to class-I MHC molecules α3 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 α 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^k 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 α antigen on Flow cytometry.

APPLICATION:
Flow cytometry; 10 µL (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:
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REFERENCES:
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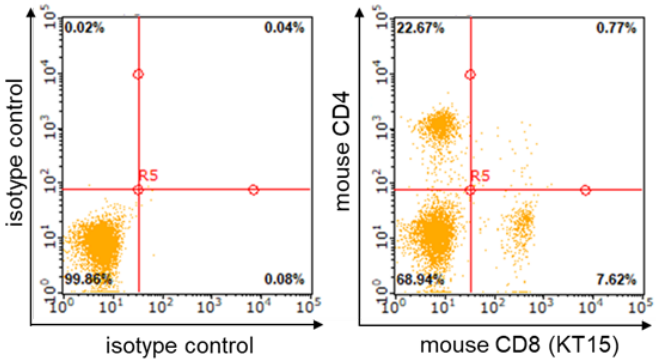
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Flow cytometric analysis of mouse CD8 expression on mouse splenocytes. *The horizontal axis shows the staining intensity of isotype control or K227-4, and the vertical axis shows the mouse CD4 staining.*

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

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_3].
   *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 10^7 cells/mL).
3) Add 50 µL 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 10 µL 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 µL 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 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse splenocyte)

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