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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-CD8 (Mouse) mAb-PE

Code No.	Clone	Subclass	Quantity
K0227-5	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 0.09% NaN_3.

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**.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

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

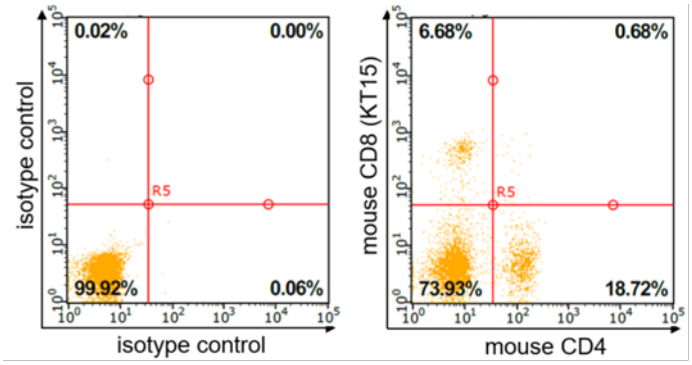
REFERENCES:
1) Whiteland, J. L., *et al*., *J. Histochem. Cytochem*. **43**, 313-320 (1995)
2) Tomonari, K., and Lovering, E., *Immunogenetics* **28**, 445-451 (1988)

Clone KT15 is used in these references.

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 (1 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 10 minutes at room temperature.
5) Add 10 µL of Anti-CD8 (Mouse) mAb-PE (KT15) 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse splenocyte)



Flow cytometric analysis of mouse CD8 expression on mouse splenocytes. *The staining intensity of K0227-5 is shown in the vertical axis with mouse CD4 staining on the horizontal axis.*

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