

MONOCLONAL ANTIBODY

# Anti-CD4 (Mouse) mAb-FITC

Code No.	Clone	Subclass	Quantity
D341-4	GK1.5	Rat IgG2b $\kappa$	1 mL (100 tests)

**BACKGROUND:** The CD4 antigen is a cell surface glycoprotein that is expressed on subsets of thymocytes and mature T lymphocytes, as well as on monocytes and macrophages. CD4 is an accessory protein for MHC class-II antigen/T-cell receptor interaction and plays an important role in T-helper cell development and activation.

This monoclonal antibody GK1.5 reacts with a non-polymorphic epitope on the mouse CD4 and a useful tool for *in vivo* depletion of CD4<sup>+</sup> T cells.

**SOURCE:** This antibody was purified from mouse ascites fluid. This hybridoma (clone GK1.5) was established by fusion of mouse myeloma cell SP2/0-Ag14 with Lewis rat splenocyte immunized with the cloned cytotoxic T lymphocyte lines V4 and 243/2.5.

**FORMULATION:** 100 tests in 1 mL volume of PBS containing 1% BSA 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.

**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 CD4 antigen on Flow cytometry.

**APPLICATION:**

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

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

**INTENDED USE:**

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

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cell	Not tested	splenocyte	Not tested
Reactivity on FCM		+	

**REFERENCES:**

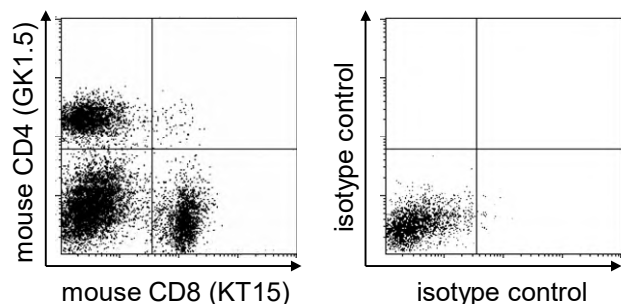
- 1) Dialynas, DP., *et al.*, *Immunol Rev.* **74**: 29-56 (1983)
- 2) Dialynas, DP., *et al.*, *J. Immunol.* **131**: 2445-2451 (1983)
- 3) Rice, JC. and Bucy, RP., *J. Immunol.* **154**: 6644-6654 (1995)
- 4) Buller, RM., *et al.*, *Nature* **328**: 77-79 (1987)
- 5) Ye, X., *et al.*, *Gene Ther.* **7**: 1761-1767 (2000)
- 6) Li, J., *et al.*, *Cancer Res.* **63**: 8384-8392 (2003)
- 7) Janssen, EM., *et al.*, *Nature* **434**: 88-93 (2005)
- 8) Moro, K., *et al.*, *Nature* **463**: 540-544 (2010)
- 9) Murooka, TT., *et al.*, *Nature* **490**: 283-287 (2012)

As clone GK1.5 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

**RELATED PRODUCTS:**

Please visit our web site <https://ruo.mbl.co.jp/>.

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



**Flow cytometric analysis of mouse CD4 expression (left) and isotypic control (right) on mouse splenocytes.** The staining intensity of D341-4 is shown in the vertical axis with mouse CD8 (clone KT15, MBL PN D271-A64) 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 0.05% NaN<sub>3</sub>].

- 2) Resuspend the cells with washing buffer ( $2 \times 10^7$  cells/mL).
- 3) Add 50  $\mu$ L of the cell suspension into each tube, and centrifuge at 400 x g for 5 minutes at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 10  $\mu$ L of Clear Back (human Fc receptor blocking reagent, MBL PN MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 80  $\mu$ L of washing buffer containing 10  $\mu$ L of the anti-CD4 (Mouse) mAb-FITC (clone GK1.5) and 10  $\mu$ L of the Alexa Fluor<sup>®</sup> 647 labeled mouse CD8 (clone KT15) to each tube. Mix well and incubate for 30 minutes at 4°C.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 400 x g for 5 minutes 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 splenocytes)