

MONOCLONAL ANTIBODY

Anti-CD52 (CAMPATH-1) (Mouse) mAb-FITC

Code No.	Clone	Subclass	Quantity	Concentration
D204-4	BTG-2G	Rat IgG2a κ	100 μ L	500 μ g/mL

BACKGROUND: CAMPATH-1, also known as CD52, is a heavily glycosylated, GPI-anchored protein expressed at high levels on almost all thymocytes, lymphocytes, monocytes, and macrophages. CAMPATH-1 is an exceptionally good target for complement-mediated cell lysis and antibody-mediated cellular cytotoxicity. Humanized CAMPATH-1 antibodies have been used therapeutically to effectively deplete lymphocytes in allogeneic bone marrow transplants, hematologic malignancies, and autoimmune diseases. Clinical trials suggest CAMPATH-1 antibodies are especially promising in the treatment of leukemia, non-Hodgkin lymphomas, and rheumatoid arthritis. The apparent size of CAMPATH-1 by SDS-PAGE is 25-29 kDa; however, the actual molecule is much smaller, ~ 8-9 kDa as confirmed by total structure analysis and mass spectrometry.

SOURCE: This antibody was purified from hybridoma (clone BTG-2G) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wistar rat splenocyte immunized with mouse IL-2R α -transgenic mouse splenocyte.

FORMULATION: 50 μ g IgG in 100 μ L volume of PBS containing 1% BSA and 0.1% ProClin150.

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 CD52 (CAMPATH-1) on Flow cytometry.

APPLICATION:

Flow cytometry: 20 μ g/mL (final concentration)

*Please refer to the data sheet (MBL, code no. D204-3) for other applications.

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		+	

REFERENCE:

1) Kubota, H., *et al.*, *J. Immunol.* **145**, 3924-3931 (1990)

Clone BTG-2G is used in this reference.

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

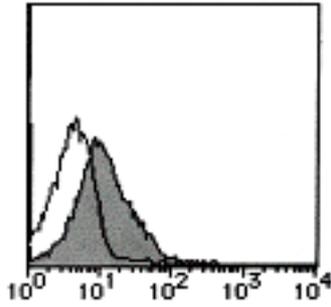
PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃].
*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 (5 x 10⁶ cells/mL).
- 3) Add 50 μ 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 10 μ 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 30 μ L of the primary antibody at the concentration of as suggested in the **APPLICATION** diluted in the washing buffer. Mix well and incubate for 20 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse splenocyte)



Flow cytometric analysis of mouse CD52/CAMPATH-1 expression on mouse splenocytes. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D204-4 to the cells.

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