

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity	Concentration
D204-3	BTG-2G	Rat IgG2a	100 μ L	1 mg/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. The function of CD52 is unknown. However, CD52 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 mouse ascites fluid using caprylic acid clarification and ammonium sulfate precipitation. This hybridoma (clone BTG-2G) was established by fusion of mouse myeloma cell P3U1 with Wister rat splenocyte immunized with mouse IL-2R alpha transgenic mice splenocytes.

FORMULATION: 100 μ g IgG in 100 μ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse CD52 (CAMPATH-1) on Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended

Immunoprecipitation; 20 μ g/1 mL of cell extract from 5x10⁶ cells

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow Cytometry; 10 μ g/mL (final concentration)

Function; Not tested*

*It is reported that this antibody can be used in this application in the reference number 1).

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

INTENDED USE:

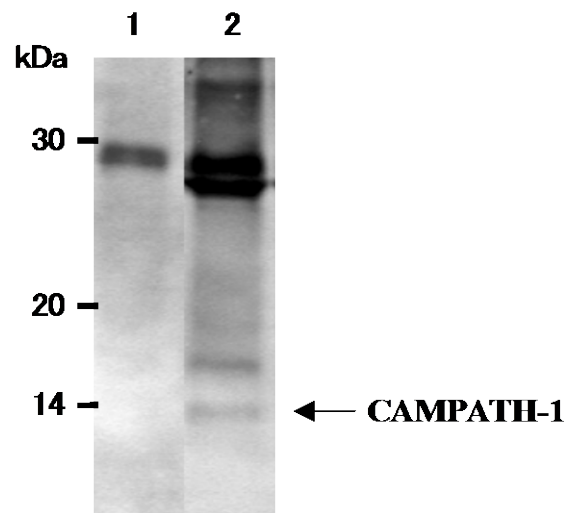
For research use only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

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

REFERENCES:

- 1) Liu, J., *et al.*, *Clin. Biol. Pharm. Bull.* **41**, 1423-1429 (2018) [Function]
- 2) Umeda, M., *et al.*, *Clin. Immunol.* **187**, 50-57 (2018) [FCM]
- 3) Sakuragi, J., *et al.*, *Microbes Infect.* **10**, 396-404 (2008) [FCM]
- 4) Kubota, H., *et al.*, *J. Immunol.* **145**, 3924-3931 (1990)



Immunoprecipitation of Mouse CAMPATH-1 from Mouse splenocytes with rat IgG2a (1) or D204-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.

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

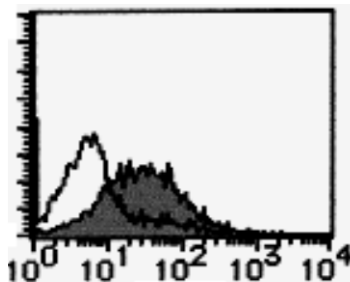
PROTOCOLS:

Immunoprecipitation

- 1) Wash the Biotin labeled mouse splenocytes 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2

- mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
 - 3) Add primary antibody as suggest in the **APPLICATIONS** into 1 mL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
 - 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
 - 5) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
 - 6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
 - 7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
 - 8) Incubate the membrane with 1:10,000 of HRP-conjugated streptavidin diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
 - 9) Wash the membrane with PBS (5 minutes x 6 times).
 - 10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
 - 11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for immunoprecipitation; Mouse splenocyte)



Flow cytometric analysis of Mouse CAMPATH-1/CD52 expression on Mouse splenocytes. Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of D204-3 to the cells.

Flow cytometric analysis for floating cells **Protocol 1**

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 (5x10⁶ 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. n
- 5) Add 30 µL of primary antibody diluted with the washing buffer as suggested in the **APPLICATIONS**. 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) Add 20 µL of 1:100 FITC conjugated anti-rat IgG (Pharmingen: code no.554016) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 8) 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.
- 9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Protocol 2

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].
- 2) Resuspend the cells with Clear Back (MBL; code no. MTG-001) (5x10⁶ cells/mL).
- 3) Add 30 µL of primary antibody diluted with the washing buffer as suggested in the **APPLICATIONS** into each tube.
- 4) Add 50 µL of the cell suspension into each tube. Mix well and incubate for 30 minutes at room temperature.
- 5) 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.
- 6) Resuspend the cells with 50 µL of the washing buffer.
- 7) Add 30 µL of 1:100 FITC conjugated anti-rat IgG (Pharmingen: code no.554016) diluted with the washing buffer into each tube. Mix well and incubate for 15 minutes at room temperature.
- 8) 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.
- 9) 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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