

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity	Concentration
D204-3	BTG-2G	Rat IgG2a	100 µL	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 µg IgG in 100 µL 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; Can be used

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow Cytometry; 10 µg/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:**

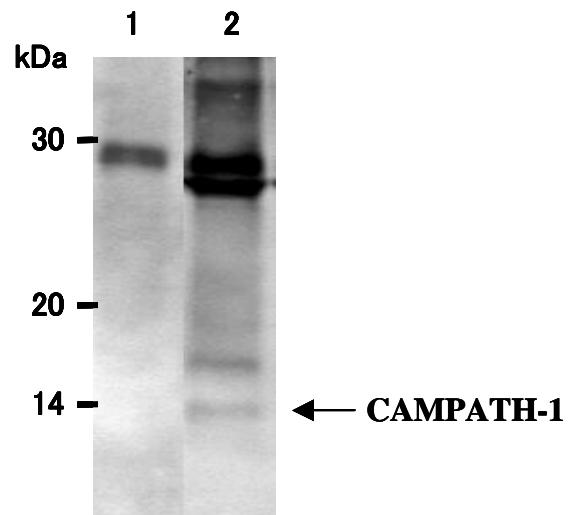
For research use only. Not for use in diagnostic procedures.

**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.

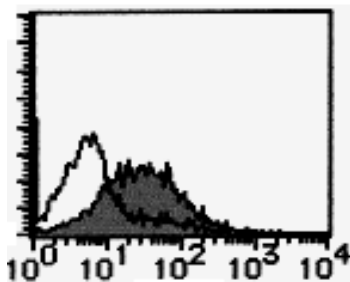
**PROTOCOLS:**

**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/cm<sup>2</sup> 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% 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.
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> 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% NaN<sub>3</sub>].
- 2) Resuspend the cells with Clear Back (MBL; code no. MTG-001) (5x10<sup>6</sup> 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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