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

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-1antibodies 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**.

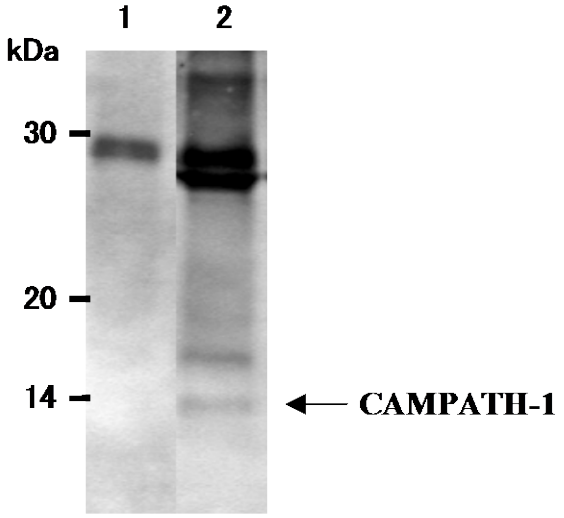
**INTENDED USE:**
For research use only. Not for use in diagnostic procedures.

**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.

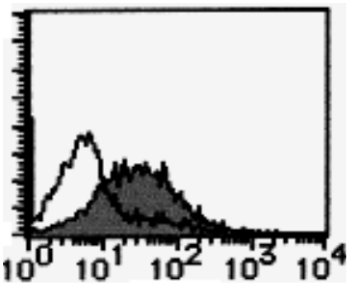
**PROTOCOLS:**

**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$^2$ 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% $NaN_3$].
*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 ($5x10^6$ 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% $NaN_3$].
2) Resuspend the cells with Clear Back (MBL; code no. MTG-001) ($5x10^6$ 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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