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MONOCLONAL ANTIBODY

Anti-CD157 (BST-1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D036-3	RF3	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: In the bone marrow (BM) hematopoietic microenvironment, stromal cells play crucial roles. Stromal cells secrete a variety of cytokines and express cell surface molecules that regulate the growth of hematopoietic cells. BM stromal cell lines derived from patients with rheumatoid arthritis (RA) have augmented ability to support the growth of a murine pre-B cell line, DW34. BST-1 (bone marrow stromal cell antigen-1) is expressed on BM stromal cell lines and is responsible for an augmented ability to support pre-B cell line growth. BST-1 is also expressed by RA-derived synovial cell lines, a myelomonocytic cell lines, and HUVEC, suggesting that BST-1 has other functional roles than just supporting pre-B cell growth.

SOURCE: This antibody was purified from hybridoma (clone RF3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3X63Ag8.653 with Balb/c mouse splenocyte immunized with RA-derived BM stromal cell line RASV5-5.

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 CD157 antigen on Flow cytometry. This antibody reacts with RASV5-5 cells, and does not react with a healthy donor-derived nonsupportive cell line, NFSV1-1.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)
Function; Not tested*
*It is reported that this antibody can be used for blocking in the reference number 2) and 3).

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

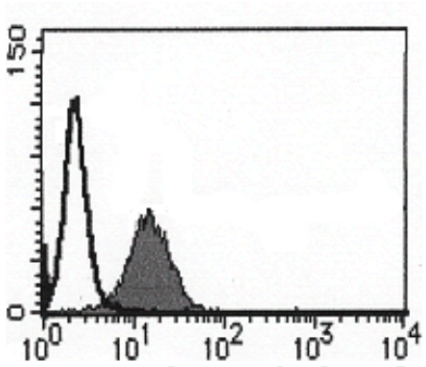
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	U937	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Ortolan, E., *et al*., *Oncotarget* **5**, 6191-6205 (2014) [FCM]
2) Morone, S., *et al*., *J. Biol. Chem*. **289**, 15588-15601 (2014) [Function]
3) Lo Buono, N., *et al*., *J. Biol. Chem*. **286**, 18681-18691 (2011) [FCM, Function]
4) Ortolan, E., *et al*., *J. Natl. Cancer Inst.* **102**, 1160-1177 (2010) [FCM]
5) Ortolan, E., *et al*., *J. Natl. Cancer Inst.* **108**, 4214-4222 (2006)
6) Ortolan, E., *et al*., *Blood* **108**, 4214-4222 (2006)
7) Funaro, A., *et al., Blood* **104**, 4269-4278 (2004)
8) Shimaoka, Y., *et al*., *J. Clin. Invest.* **102**, 606-618 (1998)
9) Kaisho, T., *et al*., *PNAS* **91**, 5325-5329 (1994)
10) Itoh, M., *et al*., *BBRC* **203**, 1309-1317 (1994)
11) Hirata, Y., *et al*., *FEBS Lett.* **356**, 244-248 (1994)

Clone RF3 is used in reference number 1) - 9).



Flow cytometric analysis of CD157 expression on U937. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D036-3 to the cells.*

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

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps 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 100 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 20 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.
5) Add 40 µL of the primary antibody at the concentration as suggest in the **APPLICATIONS** diluted in the washing buffer. 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 30 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) 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.

(Positive control for Flow cytometry; U937)

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add 50 µL of the primary antibody at the concentration as suggest in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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