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

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity	Concentration
D036-4	RF3	Mouse IgG1	100 µL	500 µg/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, 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 beads. This hybridoma was established by fusion of mouse plasmacytoma cell XAg653 with Balb/c mouse splenocyte immunized with RA-derived BM stromal cell line.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA 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.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with BST-1 on Flow Cytometry.

APPLICATIONS:
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10-20 µg/mL

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

INTENDED USE:
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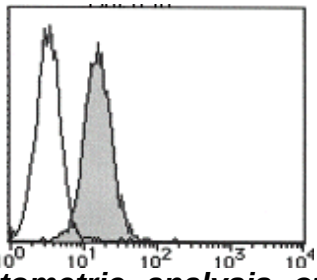
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	U937, Monocytes, Granulocytes	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:
1) Morone, S., *et al*., *J. Biol. Chem.* **289**, 15588-15601 (2014)
2) Ortolan, E., *et al*., *J. Natl. Cancer. Inst.* **102**, 1160-1177 (2010)
3) Ortolan, E., *et al*., *Blood* **108**, 4214-4222 (2006)
4) Funaro, A., *et al*., *Blood* **104**, 4269-4278 (2004)
5) Nemoto, E., *et al*., *J. Immunol.* **165**, 5807-5813 (2000)
6) Kaisho T., *et al*., *PNAS.* **91**, 5325-5329 (1994)
7) Itoh M., *et al*., *Biochem. Biophys. Res. Commun.* **203**, 1309-1317 (1994)
8) Hirata Y., *et al*. *FEBS Lett.*, **356**, 244-248 (1994)

RELATED PRODUCTS:

D036-3	Anti-CD157 (BST-1) (Human) mAb (RF3)
D036-5	Anti-CD157 (BST-1) (Human) mAb-PE (RF3)
M075-4	Mouse IgG1 (isotype control)-FITC (2E12)
MTG-001	Clear Back



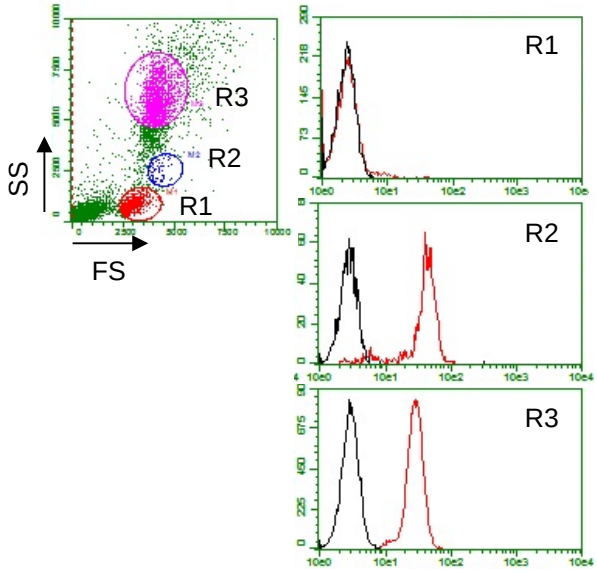
Flow cytometric analysis of BST-1 (CD157) expression on U937 cells
■: D036-4
□: isotype control (MBL; code no. M075-4)

PROTOCOLS:
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% NaN_3].
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) and 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 µL of the primary antibody as suggested 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) 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 of BST-1 (CD157) expression on Leukocytes

R1: Lymphocytes
R2: Monocytes
R3: Granulocytes
□: D036-4
□: isotype control (MBL; code no. M075-4)

Flow cytometric analysis for whole blood cells

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

1) Add 20 µL of the primary antibody as suggested in the **APPLICATIONS** diluted in the washing buffer.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
3) 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.
4) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
5) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Add 1 mL of washing buffer folled by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.