

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity
D036-5	RF3	Mouse IgG1	1 mL (50 tests)

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 plasmacytoma cell XAg653 with Balb/c mouse splenocyte immunized with RA-derived BM stromal cell line.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA 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.

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:

Western blotting; Not tested

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; 20 µL (ready for use)

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

INTENDED USE:

For research use only. Not for clinical diagnosis.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Monocyte Granulocyte	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:

- 1) Kaisho, T., *et al.*, *PNAS* **91**, 5325-5329 (1994)
- 2) Itoh, M., *et al.*, *Biochem.Biophys.Res.Comm.* **203**, 1309-1317 (1994)
- 3) Hirata, Y., *et al.*, *FEBS Lett.* **356**, 244-248 (1994)

RELATED PRODUCTS:

D036-3 Anti-CD157 (BST-1) (Human) mAb (RF3)

D036-4 Anti-CD157 (BST-1) (Human) mAb-FITC (RF3)

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 0.1% NaN₃].
- 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 normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add the primary antibody 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

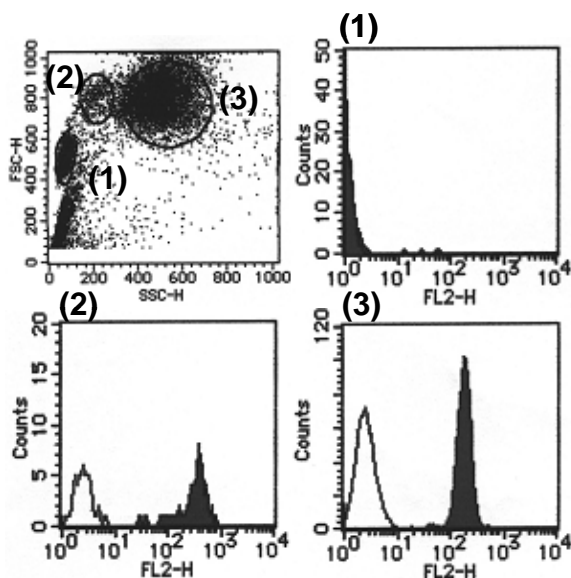
Flow cytometric analysis for whole blood cells

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

- 1) Add the primary antibody as suggested in the **APPLICATIONS** to a reaction 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 1mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) 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.
- 5) Add 1mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for flow cytometry: monocyte, granulocyte)



Flow cytometric analysis of BST-1 expression on Lymphocyte (1), Monocyte (2) and Granulocyte (3).

Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D036-5 to the cells.