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

MONOCLONAL ANTIBODY

Anti-CD257 (BAFF/BLyS) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D200-3	1D6	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: CD257/BAFF (B cell-activating factor belonging to the TNF family) is a membrane protein expressed by dendritic cells, monocytes, macrophages, follicular dendritic cells, activated T cells, activated neutrophiles, and malignant B cells. CD257/BAFF, also known as BLyS (B lymphocyte stimulator), is a potent B cell growth factor. Proteolytic cleavage can result in the release of a soluble trimeric BAFF which binds to the BAFF receptor (BAFF-R/CD268/BR3), BCMA and TACI. The release of soluble BAFF is regulated by IFN-γ and to a lesser extent IL-10. BAFF protects B cells from apoptosis and increases the expression of anti-apoptotic proteins Bcl-2 and Bcl-xL.

SOURCE: This antibody was purified from hybridoma (clone 1D6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with BALB/c mouse splenocyte immunized with human CD257 (BAFF/BLyS) transfectant.

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 human CD257 (BAFF/BLyS) on Western blotting, Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested *
*It is reported that clone 1D6 can be used in Immunohistochemistry in the reference number 1).
Immunocytochemistry ; 5 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Flow cytometry; 5 µg/mL (final concentration)

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

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

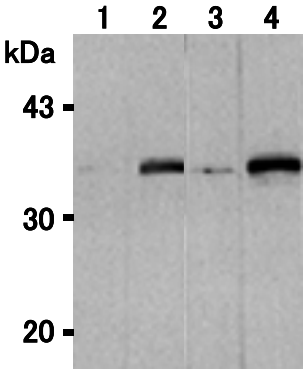
SPECIES CROSS REACTIVITY:

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

REFERENCE:

1) Hase, H., *et al.*, *Blood* **103**, 2257-2265 (2004) [WB, IHC, FCM]

Clone 1D6 is used in this reference.



Western blot analysis of BAFF/BLyS expression in mock transfected cells (1, 3) and BAFF/BLyS transfected cells (2, 4) using D200-3 at 1 µg/mL (1, 2) and 5 µg/mL (3, 4).

PROTOCOLS:

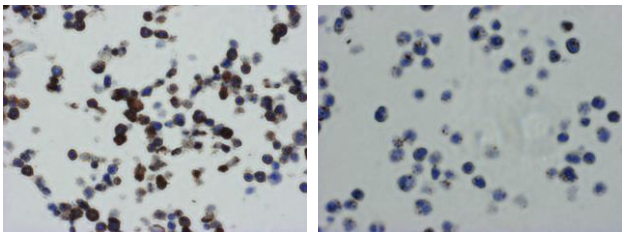
SDS-PAGE & Western Blotting

1) Wash the cells 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. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
5) 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.
6) 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.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 10% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) 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.
12) 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 Western blotting; transfectant)

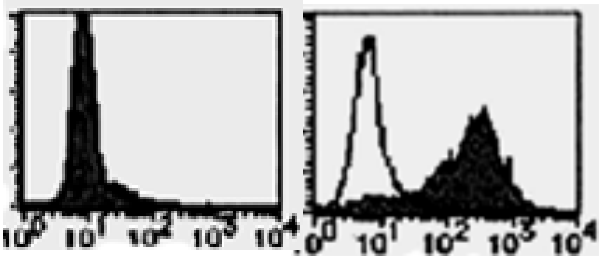


Immunocytochemical detection of BAFF/BLyS on paraffin embedded section of BAFF/BLyS transfected cells (left) and mock transfected cells (right) with D200-3.

Immunocytochemical staining for paraffin embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by microwave oven:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunohistochemistry; transfectant)



Flow cytometric analysis of BAFF/BLyS expression on BAFF/BLyS transfected cells (right) and parental cells (left). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D200-3 to the cells.*

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% 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 normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 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 with 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 1:40 anti-IgG (Mouse) pAb-FITC (MBL: code no. IM-0819) 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; transfectant)

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all step 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 0.1% 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 1:40 anti-IgG (Mouse) pAb-FITC (MBL: code no. IM-0819) 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.

(Positive control for Flow cytometry; transfectant)

RELATED PRODUCTS:

D200-3	Anti-CD257 (BAFF/BLyS) (Human) mAb (1D6)
D200-4	Anti-CD257 (BAFF/BLyS) (Human) mAb-FITC (1D6)
D201-3	Anti-CD268 (BAFF-R/BR3) (Human) mAb (8A7)
D201-4	Anti-CD268 (BAFF-R/BR3) (Human) mAb-FITC (8A7)
K0029-3	Anti-CD137 (Human) mAb (4B4-1)
K0029-4	Anti-CD137 (Human) mAb-FITC (4B4-1)
K0030-3	Anti-CD137L (Human) mAb (5F4)
K0030-4	Anti-CD137L (Human) mAb-FITC (5F4)
K0031-3	Anti-HVEM mAb (122)
K0031-4	Anti-HVEM mAb-FITC (122)
K0039-3	Anti-CD120a (TNF-R1) (Human) mAb (H398)
K0039-4	Anti-CD120a (TNF-R1) (Human) mAb-FITC (H398)
K0040-3	Anti-CD120b (TNF-R2) (Human) mAb (80M2)
K0040-4	Anti-CD120b (TNF-R2) (Human) mAb-FITC (80M2)
K0145-3	Anti-CD30 (Human) mAb (Ber-H2)
K0145-4	Anti-CD30 (Human) mAb-FITC (Ber-H2)
MD-10-3	Anti-Fas (CD95) (Human) mAb (UB2)
MD-10-4	Anti-Fas (CD95) (Human) mAb-FITC (UB2)
MD-10-5	Anti-Fas (CD95) (Human) mAb-PE (UB2)
MD-11-3	Anti-Fas (CD95) (Human) mAb (ZB4)
SY-001	Anti-Fas (CD95) mAb (CH-11)
D041-3	Anti-Fas Ligand (CD178) (Human) mAb (4H9)
D041-4	Anti-Fas Ligand (CD178) (Human) mAb-FITC (4H9)
D041-5	Anti-Fas Ligand (CD178) (Human) mAb-PE (4H9)
D041-6	Anti-Fas Ligand (CD178) (Human) mAb-Biotin (4H9)
D042-3	Anti-Fas Ligand (CD178) (Human) mAb (4A5)
D051-3	Anti-CD154 (CD40 Lgand) (Human) mAb (5F3)
D051-4	Anti-CD154 (CD40 Lgand) (Human) mAb-FITC (5F3)
D113-3	Anti-TNFα (Human) mAb (#1)
D114-3	Anti-TNF-β (Lymphotoxin) (Human) mAb (#1)
D125-3	Anti-CD134 (OX40) (Human) mAb (W4-3)
D126-3	Anti-CD252 (OX40L) (Human) mAb (TAG-34)