

**AM26519AF-N****Monoclonal Antibody to CD268 / BAFFR - Azide Free**

<b>Alternate names:</b>	B cell-activating factor receptor, BAFF receptor, BAFF-R, BlyS receptor 3, BR3, TNFRSF13C, Tumor necrosis factor receptor superfamily member 13C
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	1.0 mg/ml
<b>Background:</b>	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 neutrophils, and malignant B cells. 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-R/BR3, BCMA and TACI. BAFF-R/BR3 is the principal receptor for B cell survival and responses induced by BAFF.
<b>Uniprot ID:</b>	<a href="#">Q96RI3</a>
<b>NCBI:</b>	<a href="#">NP_443177.1</a>
<b>GeneID:</b>	<a href="#">115650</a>
<b>Host / Isotype:</b>	Mouse / IgG2a
<b>Clone:</b>	8A7
<b>Immunogen:</b>	Human BAFF-R/BR3 transfectant
<b>Format:</b>	<b>State:</b> Liquid Ig fraction <b>Purification:</b> Protein A agarose <b>Buffer System:</b> PBS containing 50% glycerol, pH 7.2. Contains no preservatives.
<b>Applications:</b>	<b>Western blot:</b> 1 µg/ml. <b>Immunohistochemistry on paraffin sections:</b> 5 µg/ml; Heat treatment is necessary; Microwave oven; 2 times for 10 minutes each in citrate buffer (pH 6.5). <b>Flow cytometry:</b> 5 µg/mL (final concentration). For details see protocols below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
<b>Specificity:</b>	This antibody reacts with human BAFF-R/BR3.
<b>Add. Information:</b>	This product was originally produced by MBL International.
<b>Storage:</b>	Upon receipt, store (in aliquots) at -20 °C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.
<b>General Readings:</b>	1. Mackay, F., et al., Annu. Rev. Immunol. 21, 231-264 (2004).
<b>Protocols:</b>	SDS-PAGE & Western Blotting 1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 oC 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 oC and transfer the

- supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make an 8 mg/mL solution.
- 3) Mix the sample with an equal volume of Laemmli's sample buffer.
  - 4) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
  - 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> 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 specific transfer procedure.
  - 6) To reduce nonspecific binding, soak the membrane in 10% Skimmed Milk in PBS for 1 hour at room temperature, or overnight at 4 oC.
  - 7) Incubate the membrane with the anti-human BAFF-R/BR3 monoclonal antibody (8A7) diluted with PBS, pH7.2 containing 1% Skimmed Milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
  - 8) Wash the membrane with PBS (5 minutes x 6 times).
  - 9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% Skimmed Milk (in PBS, pH 7.2) for 1 hour at room temperature.
  - 10) Wash the membrane with PBS (5 minutes x 6 times).
  - 11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
  - 12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The conditions for exposure and development may vary.
- Positive control for Western blotting; transfectant

#### Flow cytometric analysis for floating cells

##### Protocol 1

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 0.1% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 3) Add 50  $\mu$ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
- 4) Add 10  $\mu$ L of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN<sub>3</sub> to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25 oC).
- 5) Add 30  $\mu$ L of the anti-human BAFF-R/BR3 monoclonal antibody (8A7) (5  $\mu$ g/mL) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature (20~25 oC).
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
- 7) Add 30  $\mu$ L of 1:40 FITC conjugated anti-mouse IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature (20~25oC).
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.

9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

Positive control for flow cytometry; transfectant

#### Protocol 2

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 0.1% NaN<sub>3</sub>].
- 2) Resuspend the cells with PBS containing 25% normal goat serum, 1 mg/mL normal human IgG and 0.1% NaN<sub>3</sub> (5x10<sup>6</sup> cells/mL).
- 3) Add 30  $\mu$ L of the anti-human BAFF-R/BR3 monoclonal antibody (8A7) (50  $\mu$ g/mL) diluted with the washing buffer into each tube.
- 4) Add 50  $\mu$ L of the cell suspension into each tube. Mix well and incubate for 30 minutes at room temperature (20~25 oC).
- 5) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
- 6) Resuspend the cells with 50  $\mu$ L of the washing buffer.
- 7) Add 30  $\mu$ L of 1:40 FITC conjugated anti-mouse IgG diluted with the washing buffer into each tube. Mix well and incubate for 15 minutes at room temperature (20~25oC).
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500  $\mu$ L 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 steps described below.

- 1) Add 20  $\mu$ L of the anti-human BAFF-R/BR3 monoclonal antibody (8A7) (5  $\mu$ g/mL) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>] into each tube.
- 2) Add 50  $\mu$ L of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
- 3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
- 4) Add 30  $\mu$ L of 1:40 FITC conjugated anti-mouse IgG diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature (20~25oC).
- 5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25oC). 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 1ml of H<sub>2</sub>O to each tube and incubate for 10 minutes at room temperature (20~25 oC).
- 8) Centrifuge at 500 x g for 1 minute at room temperature (20~25oC). 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 (20~25°C). Remove supernatant by careful aspiration.

10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Immunohistochemical 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 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<sub>2</sub>O<sub>2</sub> 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 for 5 minutes to block non-specific antibody staining. Do not wash.

7) Tip off the blocking buffer, wipe gently around each section and cover tissues with the anti-human BAFF-R/BR3 monoclonal antibody (8A7) diluted with PBS containing 1% BSA as suggested 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. Incubate for 10 minutes at room temperature. Wash as in step 9).

11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. 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<sub>2</sub>O<sub>2</sub> in 150 mL PBS.

\*DAB is a suspected carcinogen and must be handled with care. Always wear gloves.

13) Wash the slides in water for 5 minutes.

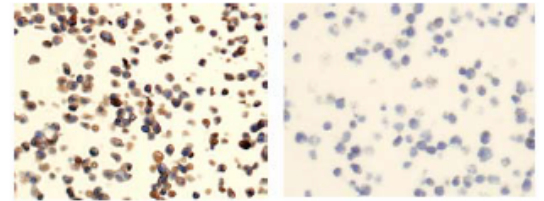
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.

15) Now ready for mounting.

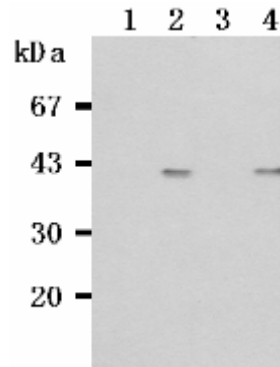
Positive control for Immunohistochemistry; transfectant

**Pictures:**

Immunohistochemical detection of BAFF-R/BR3 on paraffin embedded section of BAFF-R/BR3 transfected cells (left) and mock transfected cells (right) with AM26519AF-N.



Western blot analysis of BAFF-R/BR3 expression in mock transfected cells (1, 3) and BAFF-R/BR3 transfected cells (2, 4) using AM26519AF-N at 1 µg/ml (1, 2) and 5 µg/ml (3, 4).



Flow cytometric analysis of BAFF-R/BR3 expression on transfected cells (left). Open histograms indicate the reaction of isotopic control to the cells. Shaded histograms indicate the reaction of AM26519AF-N to the cells.

