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

MONOCLONAL ANTIBODY

Anti-GP2 (Glycoprotein 2) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D278-3	2F11-C3	Rat IgG2a κ	100 µL	1 mg/mL

BACKGROUND: M cells are located in the follicle associated epithelium (FAE) of Peyer's patches (PPs) in the small intestine, where they mediate the uptake and transcytosis of luminal antigens to the underlying lymphoid tissue. Glycoprotein 2 (GP2) is a GPI-anchor protein that was previously thought to be exclusively expressed by pancreatic acinar cells of most mammals. Recent studies reported that GP2 is specifically expressed in M cells among intestinal epithelial cells, and serves as a transcytotic receptor for mucosal antigens. Although UEA-1 (*Ulex europaeus* agglutinin-1) lectin has been widely applied to detect M cells, this lectin also stains goblet cells. Furthermore, UEA-1 can only bind mouse, but not human, M cells. Thus, GP2 is the first universal M-cell marker in mouse and human. Mouse and human GP2 recombinant proteins selectively bind a subset of commensal and pathogenic enterobacteria, including *Escherichia coli* and *Salmonella* spp., by recognizing FimH-expressing type-1-pili on the bacterial outer membrane. GP2 expressed on apical surface of M cells serves as a receptor for type-1-piliated bacteria, and facilitates translocation of these bacteria from the intestinal lumen to lymphoid follicles. Consequently, antigen-specific mucosal immune response to these bacteria is induced. Thus, GP2 plays an important role in mucosal immunosurveillance. Given that M cell are considered a promising target for oral vaccination against diverse infectious diseases, the GP2-dependent transcytotic pathway could provide a new target for development of M-cell-targeted mucosal vaccines.

SOURCE: This antibody was purified from hybridoma (clone 2F11-C31) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat lymphocyte immunized with recombinant mouse GP2-human IgG Fc fusion protein deleting transmembrane region.

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 mouse GP2 on Immunohistochemistry, Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; Not tested
Immunohistochemistry; 2.5 µg/mL
Immunocytochemistry; 2 µg/mL
Flow cytometry; 2 µg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissues	Peyer's patches	Peyer's patches	Not tested
Reactivity on IHC	-	+	

REFERENCES:

1) Lapthorne, S., *et al., Immunology* **136**, 312-324 (2012)
2) Hase, K., *et al., Nature* **462**, 226-230 (2009)
3) Terahara, K., *et al., J. Immunol.* **180**, 7840-7846 (2008)

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

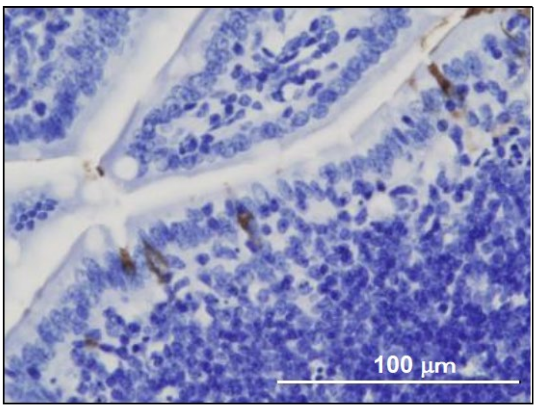
PROTOCOLS:

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 3 times in PBS-T [0.05% Tween-20 in PBS] for 3-5 minutes each.
4) Remove the slides from PBS-T, wipe gently around each section and cover tissues with 0.5% blocking reagent in PBS (Parkin Elmer) for 30 minutes to block non-specific staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 0.5% blocking reagent in PBS as suggested in the **APPLICATIONS**.
6) Incubate the sections overnight at 4°C.
7) Wash the slides 3 times in PBS-T for 5 minutes each.

8) Remove the slides from PBS-T and cover each section with 3% H_2O_2 for 20 minutes at room temperature to block endogenous peroxidase activity. Wash 2 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Histostar™ (Rat) for Mouse tissue (MBL; code no. 8463). Incubate for 1 hour at room temperature. Wash as in step 7).
10) Visualize by reacting for 10 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) 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.
13) Now ready for mounting.

(Positive control for Immunohistochemistry; Balb/c mouse Peyer's patches)



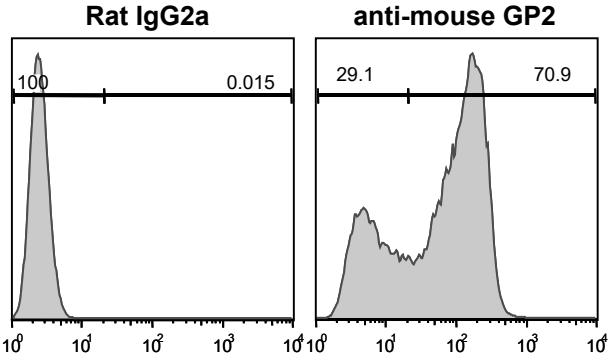
Immunohistochemical detection of mouse GP2 on paraffin embedded section of Balb/c mouse Peyer's patches with D278-3.

Flow cytometric analysis

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

1) Wash the transfectant cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% 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 ($4\text{-}6x10^5$ cells/mL).
3) Add 400 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 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30-60 minutes at 4°C.
5) 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.
6) Add 30 µL of 1:500 Alexa Fluor® 488 conjugated anti-rat IgG (Thermo Fisher Scientific; code no. A-11006) diluted with the washing buffer. Mix well and incubate for 30 minutes at 4°C.
7) 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.
8) 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 of mouse GP2 expression on mouse GP2 transfectant. *Left histogram indicates the reaction of isotypic control to the cells. Right histogram indicates the reaction of D278-3 to the cells.*

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