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

MONOCLONAL ANTIBODY

Anti-HLA-A2 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0186-3	BB7.2	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: Human leukocyte antigen-A2 (HLA-A2) is a class I molecule of the human major histocompatibility complex (MHC). It is a heterodimer composed of a 43 kDa α subunit that is non-covalently associated with the 12 kDa β2-microglobulin protein. HLA-A2, like other class I molecules, binds peptides from proteins degraded in the cytoplasm and plays a role in antigen presentation and interaction with $CD8^+$ T cells. HLA-A2 is the most common HLA-A allele in the Caucasian and American Indian populations (50% and ~30%, respectively) and A2 restricted T cell epitopes have been studied widely.

SOURCE: This antibody was purified from hybridoma (clone BB7.2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with papain solubilized HLA-A2.

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 HLA-A2 on Immunoprecipitation, Immunohistochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; Not tested

Immunoprecipitation; 1 µg/200 µL of cell extract from $5x10^6$ cells

Immunohistochemistry; 10 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)

Immunocytochemistry; 10 µg/mL
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Flow cytometry; 1 µg/mL (final concentration)

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

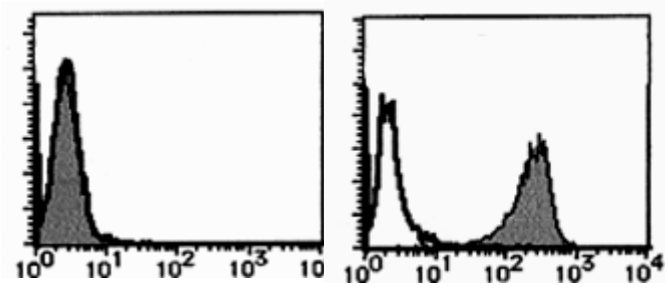
Species	Human	Mouse	Rat
Cell	T2	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:

1) Wu, H., *et al.*, *Cancer Lett.* **339**, 195-207 (2013)
2) Kozako, T., *et al.*, *J. Immunol.* **177**, 5718-5726 (2006) [FCM]
3) Yamano, Y., *et al.*, *J. Exp. Med.* **199**, 1367-1377 (2004)
4) Rodolfo, M., *et al.*, *Cancer Res.* **63**, 6948-6955 (2003)
5) Smith, M. E. F. *et al.*, *PNAS* **86**, 5557-5561 (1989)
6) Parham, P., *et al.*, *Human Immunology* **3**, 277-299 (1981)

Clone BB7.2 is used in these references.

As clone BB7.2 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.



Flow cytometric analysis of HLA-A2 expression on Jurkat cells (Left) and T2 cells (Right). *Open histogram indicates the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of K0186-3 to the cells.*

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].

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

*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.

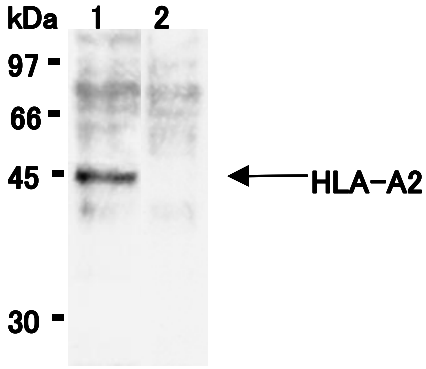
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 μL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) 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 of 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) Add FITC-conjugated anti-mouse IgG antibody 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; T2)

low 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 at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% 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 FITC-conjugated anti-mouse IgG antibody 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.



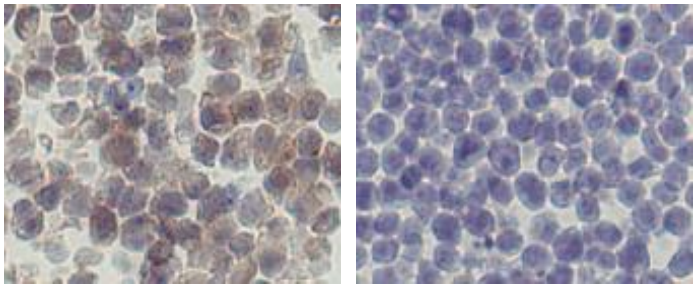
Immunoprecipitation of HLA-A2 from Biotin labeled T2 cells with K0186-3 (1) or mouse IgG2b (2) (M077-3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP conjugated Streptavidin.*

Immunoprecipitation

1) Wash the biotin labeled T2 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.
3) Add primary antibody as suggest in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the agarose 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the agarose in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) 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.
7) 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.
8) Incubate the membrane with HRP-conjugated Streptavidin diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)

9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
10) 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.
11) 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 Immunoprecipitation; T2)



Immunocytochemical detection of HLA-A2 on T2 (left) and Jurkat (right) paraffin embedded section with K0186-3.
Jurkat is a negative control.

Immunohistochemical/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; MBL, 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; T2)

RELATED PRODUCTS:

K0186-3	Anti-HLA-A2 (Human) mAb (BB7.2)
K0186-4	Anti-HLA-A2 (Human) mAb-FITC (BB7.2)
K0186-5	Anti-HLA-A2 (Human) mAb-PE (BB7.2)
K0208-3	Anti-HLA-A24 (Human) mAb (17A10)
K0208-4	Anti-HLA-A24 (Human) mAb-FITC (17A10)
K0208-5	Anti-HLA-A24 (Human) mAb-PE (17A10)
K0208-A48	Anti-HLA-A24 (Human) mAb -Alexa Fluor® 488 (17A10)
K0208-A64	Anti-HLA-A24 (Human) mAb -Alexa Fluor® 647 (17A10)
K0209-3	Anti-HLA-A24 (Human) mAb (22E1)
K0209-4	Anti-HLA-A24 (Human) mAb-FITC (22E1)
K0209-5	Anti-HLA-A24 (Human) mAb-PE (22E1)
D367-3	Anti-HLA class I (HLA-A,B,C) (Human) mAb (EMR8-5)
D370-3H	Anti-HLA class I (HLA-A,B,C) (Human) mAb (EMR8-5.1)
K0126-3	Anti-HLA-E (Human) mAb (MEM-E/02)
K0215-3	Anti-HLA-E (Human) mAb (4D12)
K0125-3	Anti-HLA-G (Human) mAb (MEM-G/1)
K0019-1	Anti-HLA-DR (Human) mAb (LN-3)
M077-3	Mouse IgG2b (isotype control) (3D12)
M077-4	Mouse IgG2b (isotype control)-FITC (3D12)
M077-5	Mouse IgG2b (isotype control)-PE (3D12)