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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-HLA class I (HLA-A,B,C) (Human) mAb

Code No.	Clone	Subclass	Quantity	Form
D370-3H	EMR8-5.1	Mouse IgG1 κ	6 mL	Ready for use

BACKGROUND: Human major histocompatibility complex (MHC) class I antigen-epitope peptide complex associated with β2-microglobulin are expressed by all human nucleated cells. It plays an important role in cell-mediated immune responses. Abnormalities in MHC class I antigen surface expression are frequently found in malignancies and infectious diseases. They are often associated with reduced recognition by MHC class I antigen-restricted, tumor or virus-associated antigen-specific cytotoxic T lymphocytes and disease progression. The EMR8-5.1 monoclonal antibody reacts with a non-polymorphic epitope of human MHC class I antigens, HLA-A, -B, and -C. This antibody is suitable for study of MHC class I down-regulation using routine formalin-fixed paraffin-embedded sections by immunohistochemistry.

SOURCE: This antibody was purified from hybridoma (clone EMR8-5.1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1with Balb/c mouse splenocyte immunized with recombinant HLA-A*24:02 extracellular domain.

FORMULATION: 6 mL volume of pre-diluted antibody in PBS, 1% BSA and 0.09% 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.

STORAGE: This antibody solution is stable for 3 years from the date of manufacture when stored at 4°C. The expiration date is indicated on the vial label.

REACTIVITY: This antibody reacts with HLA class I (HLA-A, B, C) on Immunohistochemistry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell and Tissue	Raji, Lymph node	Not tested	Not tested
Reactivity on IHC/IC	+		

INTENDED USE:
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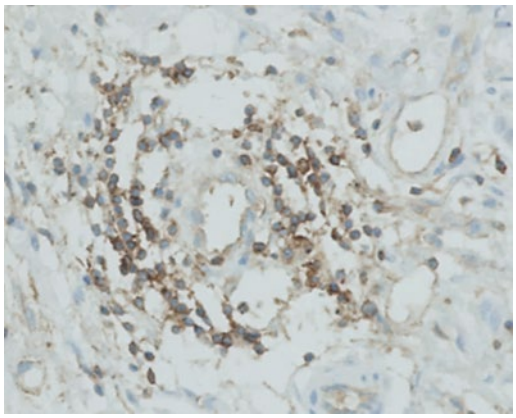
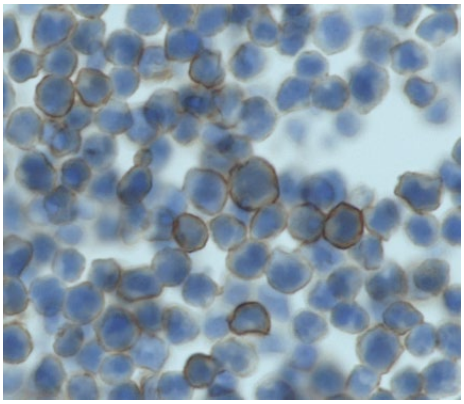
APPLICATION:
Immunohistochemistry; Ready for use
Heat treatment is necessary for paraffin-embedded sections.
Autoclave 121°C for 10 minutes in 10 mM citrate buffer (pH 6.0)

*Please refer to the data sheet (MBL; code no. D367-3) for other applications.

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



Immunohistochemical detection of HLA-A,B,C on paraffin-embedded section of lymph node with D370-3H.



Immunocytochemical detection of HLA-A,B,C on paraffin-embedded section of Raji cells with D370-3H.

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Clone EMR8-5 is used in these references.

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

PROTOCOL:

Immunohistochemical staining for paraffin-embedded sections

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 autoclave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.0). Cover the beaker with aluminum foil, and then process the slides for 10 minutes at 121°C with autoclave. 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 one or two drops of primary antibody (Ready for use).
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around the section and incubate with DAKO REAL EnVision Detection Systems Peroxidase (Agilent; code no. K500711-2). Incubate for 1 hour at room temperature. Wash as in step 8).
10) Visualize by reacting for 5 min. with DAKO REAL EnVision Detection Systems DAB Substrate Solution. *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 controls for Immunohistochemistry; Human lymph node)

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