

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

1. Materials

1.1 Reagents

- 1) Primary antibody
Anti-HLA class I (HLA-A,B,C) (Human) mAb (Clone : EMR-8-5.1)
(MBL; Code No. D370-3H, Lot No. 001)
- 2) Negative control
Mouse IgG1 Negative Control (Dako; Code No. X0931, Lot No. 20032923)
- 3) Detection kit
Bond Polymer Refine Detection (Leica; Code No. DS9800, Lot No. 60366)
<Kit components>
Peroxide block, post primary, polymer reagent, DAB chromogen, hematoxylin counterstain
- 4) Reagent for dewax, antigen retrieval and wash
 - Bond Dewax Solution (Leica; Code No. AR9222)
 - Bond Epitope Retrieval Solution 1 (Leica; Code No. AR9961)
Citrate buffer (pH6.0)
 - Bond Epitope Retrieval Solution 2 (Leica; Code No. AR9640)
EDTA based buffer (pH9.0)
 - Bond Wash Solution 10x Concentrate (for Bond RX) (Leica; Code No. AR9590)

1.2 Preparing for pathological specimen

- 1) Samples: Formalin-fixed paraffin-embedded block
- 2) Human tissues: Breast cancer, kidney cancer, colorectal cancer
- 3) Fixation: 10% neural buffered formalin

2. Procedure

Step	Vol (µL)	Name	Temperature	Time	Count
BAKING			60°C	0:30:00	
DEWAX					
Wash Slide		Bond Wash Solution			3
PEROXIDASE BLOCK					
Apply Reagent	150	Peroxidase Block			
Incubate Slide			RT	0:05:00	
Wash Slide		Bond Wash Solution			3
Antigen Retrieval					
Apply Reagent	150	BOND Epitope Retrieval Solution			
Apply Reagent	150	BOND Epitope Retrieval Solution			
Apply Reagent	150	BOND Epitope Retrieval Solution			
Apply Reagent	150	BOND Epitope Retrieval Solution			
Incubate Slide			100°C	0:20:00	
Wash Slide		Bond Wash Solution			4
Wash Slide		Bond Wash Solution		0:03:00	2
ANTIBODY					
Apply Reagent	150	Anti-HLA class I (HLA-A,B,C) (Human) mAb			
Incubate Slide			RT	0:15:00	
Wash Slide		Bond Wash Solution			3
Post Primary antibody					
Apply Reagent	150	Post Primary antibody			
Incubate Slide			RT	0:08:00	
Wash Slide		Bond Wash Solution		0:02:00	3
POLYMER HRP					
Apply Reagent	150	Polymer-HRP			
Incubate Slide			RT	0:08:00	
Wash Slide		Bond Wash Solution		0:02:00	2
Wash Slide		Deionized Water			1
DAB Working					
Apply Reagent	150	DAB Solution			
Apply Reagent	150	DAB Solution			
Incubate Slide			RT	0:10:00	
Wash Slide		Deionized Water			3
HEMATOXYLIN					
Apply Reagent	150	Hematoxylin			
Incubate Slide			RT	0:05:00	
Wash Slide		Deionized Water			1
Wash Slide		Bond Wash Solution			1
Wash Slide		Deionized Water			1