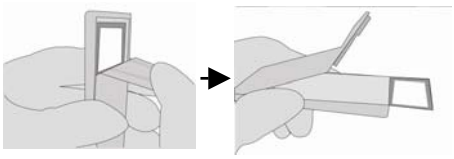
AccuMax™ Array

# Protocol



Carefully open the slide case

For FFPE tissues



For Frozen tissues



ISU ABXIS CO., LTD

AccuMax™ Array

# H&E Staining Protocol

**for formalin fixed paraffin embedded (FFPE) tissues**

For research use only

## •Deparaffinize and hydrate the tissue section

1*[1]. Put the slides into a rack
2. Dry slides at 65℃ for 1-2 hours
3. Dip the rack in 4 consecutive stain jars containing xylene to remove paraffin
   - 10 minutes every step
4. Dip the rack in ethanol to remove xylene
   - 100% Ethanol for 5 minutes
   - 95% Ethanol for 5 minutes
   - 80% Ethanol for 5 minutes
   - 70% Ethanol for 5 minutes
5*[2]. Rinse the rack with tap water to remove ethanol for 5 minutes

**Procedure 1~2.**
Slide in a rack

**Procedure 3~.**
Rack in stain jar

*[1]. Cool down the slides at RT before dipping them into xylene to prevent the possible loss of tissues.

*[2]. Prepare a container which is large enough to afford 2 racks. Put the rack at one side of the container and, then, make water flow from the other side. Don't make the tissue side face the water flow directly.

## •Stain nucleus with Hematoxylin*[1]

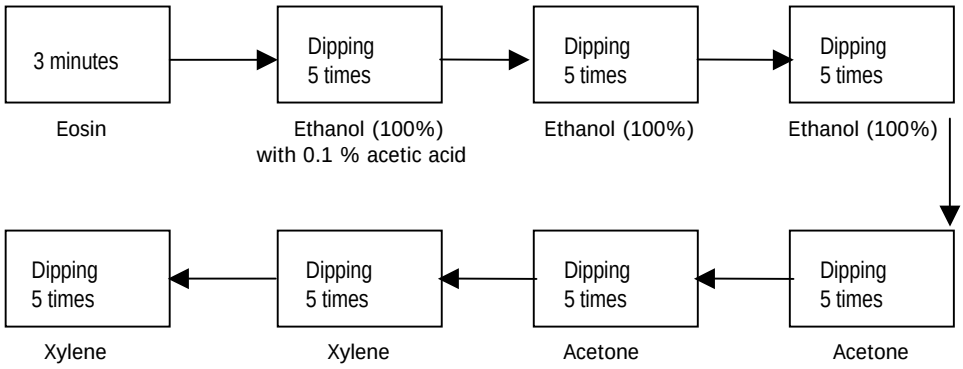
6. Put the rack into a container filled with Hematoxylin for 10 minutes*[2]
7. Rinse the rack with tap water to remove Hematoxylin for 10 minutes
8. Dip the rack into a jar containing 0.1% HCl 3 times and, then, into tap water 3~4 times
9. Dip the rack into a jar containing 0.1% $NH_4OH$ 3 times and, then, into tap water 3~4 times

*[1]. Hematoxylin must be protected from the light and filtered before use to eliminate oxidized golden sediments.

*[2]. The time of staining could be different depending on Hematoxylin. That's because Hematoxylin is diluted by repeated uses. It takes more time to stain the tissue if old Hematoxylin is used. However, there is no significant effect on the results of staining.
New Hematoxylin: about 5 minutes, old Hematoxylin (about 7 days old ): over 15 minutes.

## •Stain cytoplasm with Eosin and dehydrate

10. Dip the rack into the following solutions




## •Mounting

11. Drop 2-3 drops of mountant onto the slide and, then, put a cover glass onto the slide

# IHC (immunohistochemistry) – For FFPE tissues

Immunohistochemistry is an exquisitely sensitive method for locating an antigen within a cell or tissue through a high-resolution image (a single cell among thousands or millions). The method is based on the use of a primary antibody binding specifically to its cognate antigen. The bound antibody is then visualized through colorimetric or fluorescent detection methods.

## •Deparaffinize and hydrate the tissue section

1 *1. Put the slides into a rack for IHC

2. Dry slides at 58℃ overnight (recommended) or at 65℃ for 1-2 hours(for fast experiment)

**Procedure 1~2.**
Slide in a rack

3. Dip the rack into 4 consecutive stain jars containing xylene to remove paraffin
   - 10 minutes every step

4. Dip the rack into ethanol to remove xylene
   - 100% Ethanol 5min
   - 95% Ethanol 5min
   - 80% Ethanol 5min
   - 70% Ethanol 5min

5*2. Rinse the rack with tap water to remove ethanol for 5 minutes

**Procedure 3~.**
Rack in a stain jar

*1. Cool down the slides at RT before dipping them into xylene to prevent the possible loss of tissues.

*2. Prepare a container which is large enough to afford 2 racks. Put the rack at one side of the container and, then, make water flow from the other side. Don't make the tissue side face the water flow directly.

## •Quench the peroxidase

6. Dip the rack in 3% $H_2O_2$ for 13 minutes

7. Rinse the rack with tap water for 15 minutes

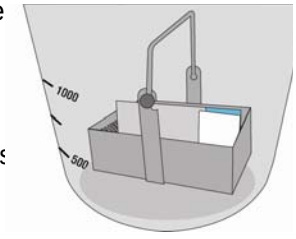
## •Retrieve antigen

8-1. Put citrate buffer*1 into a pressure cooker and heat it without a lid in the microwave for 5 minutes (Prewarming)

*1. Citrate buffer ( 0.01M citric acid, pH 6.0 ) should be stored at 4 ℃.

8-2. Put the rack into the citrate buffe

9. Put a lid on the cooker and heat the cooker in the microwave for 10~15 minutes




**Procedure 8~.**
Use beakers if a cooker is not available. Pour the buffer enough to immerse the slide rack. If there is no lid, evaporation can cause the tissues to be dried. In general, the slide should be dipped enough into the buffer, almost two times the height of the slide. As in this figure, if 1000ml beaker and 600ml of buffer are used, the volume of the buffer decreases down to 400ml because of evaporation. There is no other effects on experiment if the tissues are in the buffer and not dried.

10. Remove the cooker from the microwave and cool it down at RT for 30 minutes

11. Rinse the rack with tap water for 10 minutes

12. Dip the slides in PBS*2 buffer (at RT) for 10 minutes

*2. PBS should be stored at 4℃.

The following process could be different depending on the use of antibody (Ab). Therefore, you need to follow the protocol of a detection kit. Here, the detection kit of Zymed (CEA 18-0057) is used.

## •Primary antibody*1

13. Adjust Ab with dilution solution (refer to the protocol you use, here, Ab = CEA)

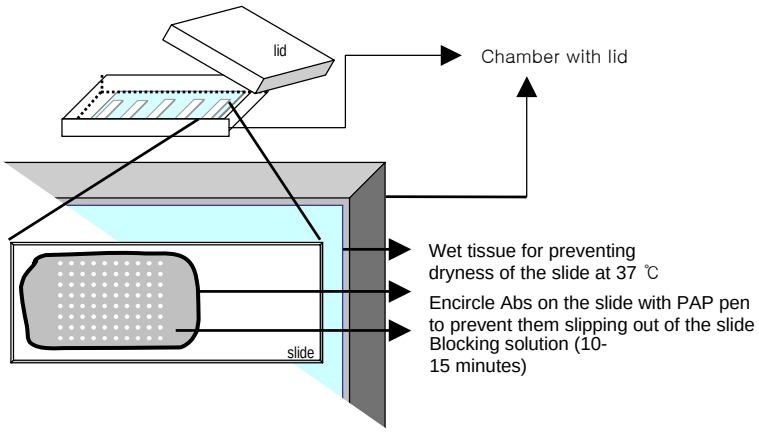
13-1. Put a wet tissue on the chamber (it should have a lid)

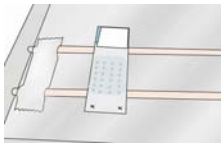
13-2*2. Encircle the tissues with a PAP pen, put the slide on the wet tissue and drop 2-3 drops of blocking solution (10-15 minutes)

13-3. Drain the blocking solution out of the slide (not washing)

*1. When you spread solution on the slide, be careful to cover tissues completely.

*2. The slide should not be dried (put the lid on the chamber).



**Procedure 13~.**
If a chamber is not available, make it with 2 pieces of 1ml pipet and a tray like th figure above. Be sure to lay wet tissues under the slide.

14. Ab = CEA, drop Ab solution enough to cover the tissue

15. Keep the chamber at 37℃ for 1-2 hours*3

16. Remove the Ab solution by spreading out PBS onto the slide well and put the slides in the rack

17. Dip the rack in PBS*4 buffer (at RT) for 10 minutes

*3. The slide should not be dried (put the lid on the chamber).

*4. PBS should be stored at 4℃.

## •Secondary antibody*

18. Put the slides in the chamber again

19. Drop 2-3 drops of biotinylated secondary antibody (Zymed) to the slide (5- 10 minutes)

20. Repeat steps 16 -18

21. Drop 2-3 drops of streptavidin-HRP to each slide (10-15 minutes)

22. Repeat steps 16-18

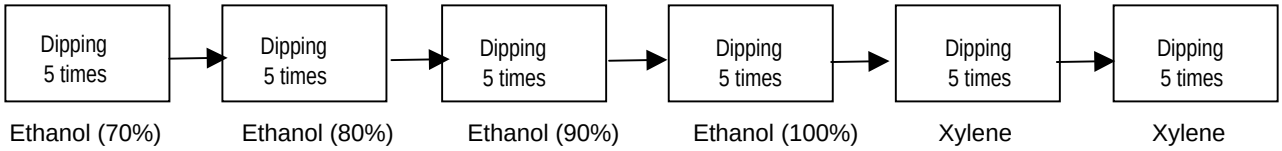
* When you spread solution on the slide, be careful to cover tissues completely.

## •Colorimetric detection

23. Mix 1 ml DW with reagents (substrate buffer, DAB concentrate, hydrogen peroxide: 1 ml/each)
24. Drop the mixture (2-3 drops) onto the slide (after 3–5 minutes, stained tissues can be observed with a microscope)
25. Wash the slide with PBS buffer when you believe the tissues are stained well enough for examination

## •Counter stain (Harris Hematoxylin)

26. Dip the slides in the rack into Hematoxylin for 5–10 minutes
27. Rinse the slides with tap water
28. Dip the rack into a jar containing 0.1% HCl 3 times and, then, into tap water 3 times
29. Dip the rack into 0.1% $NH_4OH$ 3 times and, then, into tap water 3 times
30. Dip the rack in ethanol




## •Mounting

31. Drop 2–3 drops of mountant*1 onto the slide
32*2. Put a cover glass onto the slide

*1. eg. ThermoShandon: Synthetic mountant, product # 6769007
*2. Be careful not to create vapor between the cover glass and the slide

## •Hydrate the tissue section

1. Put the slide into the 100% ethanol after tissue section
2*1. Rinse the rack with tap water to remove ethanol for 5 minutes

*1. Prepare a container which is large enough to afford 2 racks. Put the rack at one side of the container and, then, make water flow from the other side. Don't make the tissue side face the water flow directly.

## •Stain nucleus with Hematoxylin*1

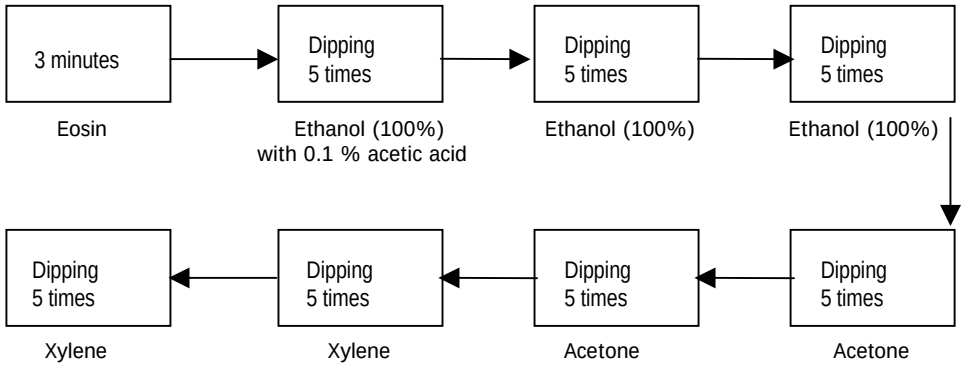
3. Put the rack into a container filled with Hematoxylin for 10 minutes*2
4. Rinse the rack with tap water to remove Hematoxylin for 10 minutes
5. Dip the rack into a jar containing 0.1% HCl 3 times
   and, then, into tap water 3~4 times
6. Dip the rack into a jar containing 0.1% $NH_4OH$ 3 times
   and, then, into tap water 3~4 times

*1. Hematoxylin must be protected from the light and filtered before use to eliminate oxidized golden sediments.

*2. The time of staining could be different depending on Hematoxylin. That's because Hematoxylin is diluted by repeated uses. It takes more time to stain the tissue if old Hematoxylin is used. However, there is no significant effect on the results of staining.
New Hematoxylin: about 5 minutes, old Hematoxylin (about 7 days old ): over 15 minutes.

## •Stain cytoplasm with Eosin and dehydrate

7. Dip the rack into the following solutions




## •Mounting

8. Drop 2-3 drops of mountant onto the slide and, then, put a cover glass onto the slide

# IHC (immunohistochemistry) – For Frozen tissues

Immunohistochemistry is an exquisitely sensitive method for locating an antigen within a cell or tissue through a high-resolution image (a single cell among thousands or millions). The method is based on the use of a primary antibody binding specifically to its cognate antigen. The bound antibody is then visualized through colorimetric or fluorescent detection methods.

## •Hydrate the tissue section

1*1. Put the slides into a rack for IHC
2. Dip the slides into the 100% cold ethanol in the 4°C refrigerator for 10minutes
3. Dry slides at RT for 20~30minutes
4 2*. Dip the slides in PBS*2 buffer (at RT) for 10 minutes

**Procedure 1.**
Slide in a rack

**Procedure 2~.**
Rack in a stain jar

*1. Prepare a container which is large enough to afford 2 racks. Put the rack at one side of the container and, then, make water flow from the other side. Don't make the tissue side face the water flow directly.

*2. PBS should be stored at 4℃.

The following process could be different depending on the use of antibody (Ab). Therefore, you need to follow the protocol of a detection kit. Here, the detection kit of Zymed (CEA 18-0057) is used.

## •Primary antibody*1

5. Adjust Ab with dilution solution (refer to the protocol you use, here, Ab = CEA)

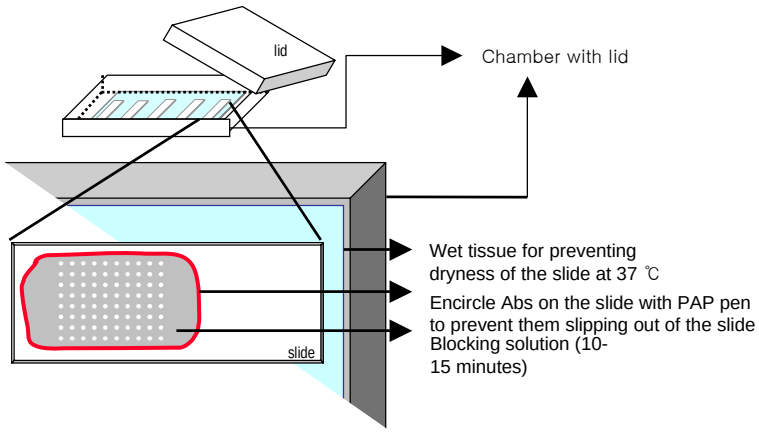
5-1. Put a wet tissue on the chamber (it should have a lid)

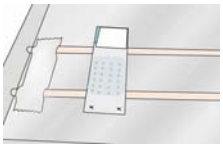
5-2*2. Encircle the tissues with a PAP pen, put the slide on the wet tissue and drop 2-3 drops of blocking solution (10-15 minutes)

5-3. Drain the blocking solution out of the slide (not washing)

*1. When you spread solution on the slide, be careful to cover tissues completely.

*2. The slide should not be dried (put the lid on the chamber).



**Procedure 5~.**
If a chamber is not available, make it with 2 pieces of 1ml pipet and a tray like th figure above. Be sure to lay wet tissues under the slide.

The following process could be different depending on the use of antibody (Ab). Therefore, you need to follow the protocol of a detection kit. Here, the detection kit of Zymed (CEA 18-0057) is used.

## •Primary antibody*1

6. Ab = CEA, drop Ab solution enough to cover the tissue
7. Keep the chamber at 37℃ for 1-2 hours*3
8. Remove the Ab solution by spreading out PBS onto the slide well and put the slides in the rack
9. Dip the rack in PBS*4 buffer (at RT) for 10 minutes

*3. The slide should not be dried (put the lid on the chamber).

*4. PBS should be stored at 4℃.

## •Secondary antibody*

10. Put the slides in the chamber again
11. Drop 2-3 drops of biotinylated secondary antibody (Zymed) to the slide (5- 10 minutes)
12. Repeat steps 16 -18
13. Drop 2-3 drops of streptavidin-HRP to each slide (10-15 minutes)
14. Repeat steps 16-18

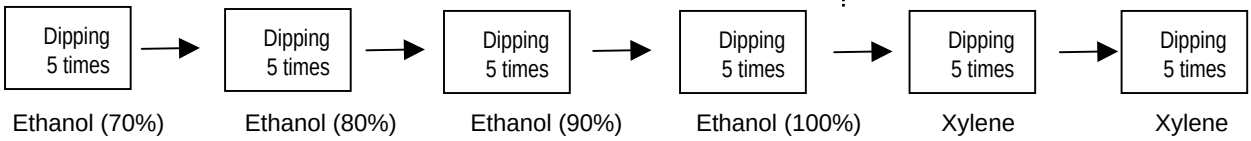
* When you spread solution on the slide, be careful to cover tissues completely.

## •Colorimetric detection

15. Mix 1 ml DW with reagents (substrate buffer, DAB concentrate, hydrogen peroxide: 1 ml/each)
16. Drop the mixture (2-3 drops) onto the slide (after 3–5 minutes, stained tissues can be observed with a microscope)
17. Wash the slide with PBS buffer when you believe the tissues are stained well enough for examination

## •Counter stain (Harris Hematoxylin)

18. Dip the slides in the rack into Hematoxylin for 5–10 minutes
19. Rinse the slides with tap water
20. Dip the rack into a jar containing 0.1% HCl 3 times and, then, into tap water 3 times
21. Dip the rack into 0.1% $NH_4OH$ 3 times and, then, into tap water 3 times
22. Dip the rack in ethanol



| •**Mounting** | |
|---|---|
| 23. Drop 2–3 drops of mountant*1 onto the slide<br>24*2. Put a cover glass onto the slide | *1. eg. ThermoShandon: Synthetic mountant, product # 6769007<br>*2. Be careful not to create vapor between the cover glass and the slide |