

•Deparaffinize and hydrate the tissue section

1. Dry slides at 65°C for 1-2 hours
- 2*1. Put the slides into a rack
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 5min
 - 95% Ethanol 5min
 - 80% Ethanol 5min
 - 70% Ethanol 5min
- 5*2. Rinse the rack with tap water to remove ethanol for 5 minutes

*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

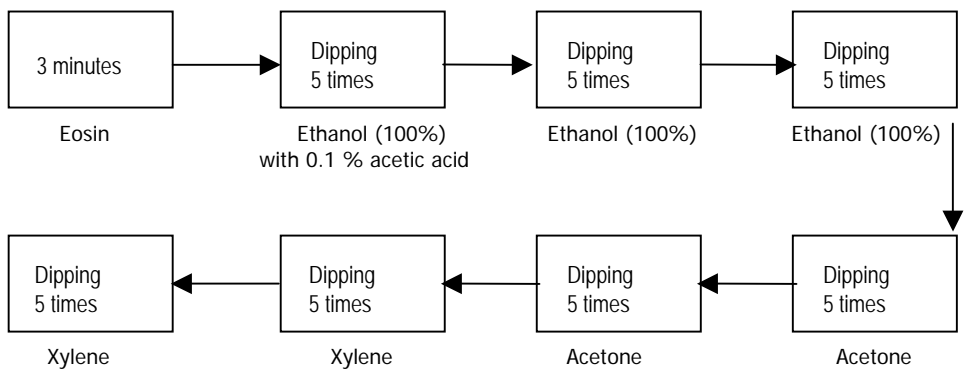
6. Put the rack into a container filled with Hematoxylin for 10 minutes*2
7. Rinse the rack with tap water to remove Hematoxylin for 10 minutes
8. Dip the rack into a jar containing 0.1% HCl 3 times and, then, into tap water 3~4 times
9. Dip the rack into a jar containing 0.1% NH₄OH 3 times and, then, 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