

Hematoxylin & Eosin Staining Protocol

Safety Precautions

ALWAYS be careful when handling solvents, they will permanently stain skin and clothing and can cause health hazards.

ALWAYS keep solvent containers closed when not in use.

NEVER dispose of Xylene, Hematoxylin, or Eosin in the sink. Use the designated waste containers in the fume hood.

Materials

1. Exam gloves
2. Timer
3. Fume hood
4. Absorbent liner
5. Water bath with drain
6. Pasteur pipette
7. Slides with mounted tissues to be stained
8. Coverslips
9. Mounting medium
10. Staining jars, at least 6 of which must be solvent-resistant for the xylene
11. 70%, 95%, and 100% alcohol
12. Bluing agent
13. Clearing agent
14. Eosin stain
15. Hematoxylin stain
16. Xylene

Procedure

1. Put an absorbent liner inside the fume hood to cover the staining station area.
2. Set up a rinsing container in the fume hood near the water supply and drain that can be filled and drained slowly and continuously during the staining procedure. This allows the reagents to be adequately rinsed without dislodging tissue sections.
3. Fill the staining jars to the fill line with the reagents named on each jar's label and line the jars up in the following order:

X, X, X, 100%, 100%, 95%, H, C, B, E, 70%, 95%, 100%, 100%, X, X, X

X = xylene

100% = 100% ethanol

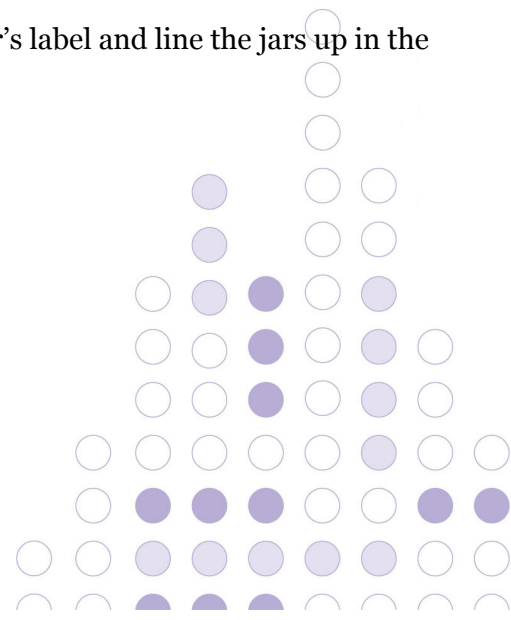
95% = 95% ethanol in distilled water

H = hematoxylin

C = clearing reagent

B = bluing reagent

70% = 70% ethanol in distilled water



4. Dip the slides for the given time in the solutions as detailed below where the first row is the solution and the second row is time in minutes (m) or seconds (s). R = water rinse.

X	X	X	100	100	95	R	H	R	C	R	B	R	E	70	95	100	100	X	X	X
3 m	3m	3 m	2 m	2 m	2 m	30 s	2m	1m	1m	30s	1m	30s	30s	30 s	30 s	15 s	15 s	3m	3 m	3 m

- After the first xylene series, you should notice the slide looking clear since the xylene is removing the paraffin.
- After each of the stains, rinse the slide until the water dripping off the slide is clear.
- Following the last immersion in xylene, lay one slide at a time on a clean flat surface with the stained section facing up. Apply enough mounting medium to cover the section and fill the space under the cover slip in step 8.
- Carefully lay a coverslip over the section allowing the flow of the mounting medium over and around the section to prevent the formation of bubbles.
- When staining is complete, be sure all containers are securely closed. Dispose of waste in the appropriate container.

