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Haematoxylin Eosin (H&E) staining

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- 1 Formaldehyde fixation: Rinse fresh colon tissue with PBS buffer, then fix tissues with fresh 4% formalin for 24 hours at room temperature. Make sure you have enough fixative to cover tissues. Fixative volume should be 5-10 times of tissue volume.
- 2 Washing: small flow water rinse formalin fixed colon tissue 30~60 minutes, rinse the fixative.
- 3 Dehydration: Process for paraffin embedding schedule as follow: 70% Ethanol, 15~30 minuate; 80% Ethanol, one change, 2~4 hours; 95% Ethanol, one change, 2~4 hours; 100% Ethanol (In order to ensure that 100% ethanol anhydrous, ethanol can be placed in the container of anhydrous copper sulfate to absorb moisture), three changes, 1.5 hour each;
- 4 Transparent: the dehydrated colon tissue is placed in xylene I solution (100% ethanol and pure fresh xylene solution 1:1) for 30 minutes, then placed in xylene II (pure fresh xylene solution) solution for 30 minutes;
- 5 Soaking wax: Because the selected wax melting point is 52~56 °C, the thermostat is set to 56 °C, filled with melted wax cup, transparent colon tissue into soft wax cup for 1 hour, then into hard wax dipping wax for another 1 hour;
- 6 Embedding tissues into paraffin blocks: put the melted hard wax into the embedding metal box, and then quickly colon tissue of hard wax cup by heating the forceps delivery placed in the box, flat bottom, and then embedding frame immediately into the cold water cooling, after about 20 minutes after the solidification of the wax embedding block two blocks; The paraffin tissue block can be stored at room temperature for years.
- 7 Trim paraffin blocks as necessary and cut at 3-10 um (5 um slice thickness is commonly used, blade angle in 20~30 degrees).
- 8 Show: Place paraffin ribbon in water bath at about 40-45 °C.
- 9 Patch: Mount sections onto glass slides, slide the glass on the 60 temperature mounter and place it for 1-2 hours to get a slice.
- 10 Prepare formalin-fixed, paraffin-embedded tissue sections
- 11 Dewaxing: paraffin sections are placed in xylene I solution and xylene II solution each for 5 minutes (56 °C in winter);
- 12 Rehydration: paraffin slices will be placed in 100%, 95%, 80% and 75% ethanol solution, each time for 3 minutes, and then rinse with distilled water for 5 minutes, dried water;



- 13 Hematoxylin staining: the paraffin sections stained with hematoxylin about 10 minutes (30 °C), water rinse for 15 minutes, drain the water;
- 14 Differentiation: put the paraffin slices into 1% hydrochloric acid ethanol differentiation liquid solution in 5~30 seconds until the slice get red, then rinse water for about 15 minutes to the section of the eye can be seen blue; check the nucleus is appropriate use microscope.

Dehydration: paraffin slices will be put into 75%, 95%, 100%, 100% ethanol solution for 5 minutes each;

15

- 16 Eosin dye re dyeing: With eosin dye staining 2 min, tap water wash for 1 min; 0.5% eosin alcohol solution for 1-2 minutes.
- 17 Dehydration: paraffin slices will be put into 95% and 100% ethanol solution for 5 minutes, then dehydrated by 95% ethanol for 2 minutes, dehydrated by 95% ethanol for 2 minutes, dehydrated by 100% ethanol for 1 minute, dehydrated by 100% ethanol for 1 minute.

Transparent: paraffin slices will be put into xylene I solution and xylene II solution each for 5 minutes;

18

- 19 Sealing glue: Wipe off the xylene off the back of a slide on a paper towel. Dripping 1-2 drops of neutral Canada gum onto the dry slice, and quickly take it cover with clean coverslip (do not leave bubbles) by tweezers. Place slides on a paper towel to cure overnight. The nucleus is blue, cytoplasm and fibrous tissue was ranging from shades of red.