# Haematoxylin Eosin (H&E) staining

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## Abstract

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## Protocol

**Step 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.

**Step 2.**
Washing: small flow water rinse formalin fixed colon tissue 3060 minutes, rinse the fixative.

**Step 3.**
Dehydration: Process for paraffin embedding schedule as follow: 70% Ethanol, 1530 minuate; 80% Ethanol, one change, 24 hours; 95% Ethanol, one change, 24 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;

**Step 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;

**Step 5.**
Soaking wax: Because the selected wax melting point is 5256 ºC, the thermostat is set to 56 ºC, 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;

**Step 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.

**Step 7.**
Trim paraffin blocks as necessary and cut at 3-10 um (5 um slice thickness is commonly used, blade angle in 2030 degrees).

**Step 8.**
Show: Place paraffin ribbon in water bath at about 40-45 ºC.

**Step 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.

**Step 10.**

Prepare formalin-fixed, paraffin-embedded tissue sections

**Step 11.**

Dewaxing: paraffin sections are placed in xylene I solution and xylene II solution each for 5 minutes (56 ºC in winter);

**Step 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;

**Step 13.**

Hematoxylin staining: the paraffin sections stained with hematoxylin about 10 minutes (30 ºC), water rinse for 15 minutes, drain the water;

**Step 14.**

Differentiation: put the paraffin slices into 1% hydrochloric acid ethanol differentiation liquid solution in 530 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%, l00% ethanol solution for 5 minutes each;

**Step 15.**

**Step 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.

**Step 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;

**Step 18.**

**Step 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.