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Hematoxylin and eosin staining on FFPE sections

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We use this protocol and it's working.

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Abstract

This protocol describes the steps for doing a manual Hematoxylin and Eosin (H&E) Stain of formalin-fixed paraffin-embedded (FFPE) tissue. This protocol is developed to utilise the reagents from the H&E staining process in 10x Genomics Visium HD workflow (CG000684).

Materials

Reagents and consumables

- Xylene, Reagent grade, Sigma 214736
- Ethanol, Pure (200 Proof, anhydrous), Sigma 534056
- Eosin Y solution, alcoholic, Sigma HT110116-500ML
- Gill II hematoxylin, Sigma GHS232
- Bluing buffer, Dako CS70230-2
- DPX mounting medium, Sigma 06522-100mL
- Coverslips

Equipment

- Chemical fume hood
- Microscope
- Easydip™ slide staining jars, Simpot M900-12
- Slide staining racks, M905-12DGY

Safety warnings

Appropriate precautions must be taken when working with xylene and ethanol. All staining procedures should be performed inside a certified fume hood to ensure proper ventilation. Nitrile gloves must be worn when handling xylene to prevent skin exposure.

Before start

- Ensure slides have been incubated in the oven for 02:00:00 at 60 °C.
- Ensure each jar has more than 80 mL of reagents for staining.

EasyDip™ slide staining jars.

Deparaffinization

37m

1 Immerse slides in Xylene Dewax Jar 1 for 00:10:00 . 10m

> Note
>
> Close Jar lid to minimise Xylene evaporation.

2 Immerse slides in Xylene Dewax Jar 2 for 00:10:00 . 10m

> Note
>
> Close Jar lid to minimise Xylene evaporation.

3 Immerse slides in 100% Ethanol Dewax Jar 1 for 00:03:00 . 3m

4 Immerse slides in 100% Ethanol Dewax Jar 2 for 00:03:00 . 3m

5 Immerse slides in 96% Ethanol Dewax Jar 1 for 00:03:00 . 3m

6 Immerse slides in 96% Ethanol Dewax Jar 2 for 00:03:00 . 3m

7 Immerse slides in 70% Ethanol Dewax Jar for 00:03:00 . 3m

8 Immerse slides in H_2O Jar for 00:02:00 . 2m

Staining and Dehydration

21m 50s

9 Immerse slides in Hematoxylin Jar for 00:01:00 1m

10 Rinse with deionized water in three H_2O jars, keep the slides in the last jar with running deionized water for at least 00:05:00 in the sink. Check the hematoxylin staining under the microscope. 5m

11 Immerse in Bluing Buffer for 00:01:00 . 1m

12 Rinse with deionized water in three H_2O jars, keep the slides in the last jar with running deionized water for at least 00:05:00 in the sink. 5m

Note

Carryover of the bluing reagent can raise the pH of eosin, resulting in a weak eosin staining.

13 Dip in 96% EtOH Before Eosin Jar for 00:00:30 . 30s

14 Immerse in Eosin Jar for 00:02:00 . 2m

15 Immerse in 96% EtOH Dehydration Jar 1 for 00:00:10 . 10s

16 Immerse in 96% EtOH Dehydration Jar 2 for 00:00:10 . 10s

17 Immerse in 100% EtOH Dehydration Jar 1 for 00:01:00 . 1m

18 Immerse in 100% EtOH Dehydration Jar 2 for 00:01:00 . 1m

19 Immerse in 100% EtOH Dehydration Jar 3 for 00:01:00 . 1m

20 Immerse in Xylene Dehydration Jar 1 for 00:02:00 . 2m

21 Immerse in Xylene Dehydration Jar 2 for 00:02:00 . 2m

22 Keep the slides in Xylene Clearing Jar. Apply DPX mounting medium onto the section, and place the coverslip onto the mounting medium slowly to avoid generating any bubble with tweezers.

Note

Remove any dust on the coverslip with an air blower.

Storage and Imaging 1d

23 Let slides dry for at least 24:00:00 for the mounting medium to cure before imaging on the slide scanner. Before scanning slides, ensure no DPX mounting medium is overhanging the slide. Scrape off excess mounting medium with a blade. 1d