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H&E Staining for 10X Genomics Visium Imaging

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes H&E staining of 10X Genomics Visium slides prior to imaging and is adapted from 10X Genomics protocol documented in "**Methanol Fixation, H&E Staining & Imaging for Visium Spatial Protocols**". H&E staining and imaging is essential to ensuring target tissue/region/cells are mounted within the fiducial frame of the Visium slide.

Materials

Eosin Mix: 1mL

- 100µL Eosin Y solution (Sigma-Aldrich, HT110216-500mL)
- 900µL Tris-Acetic Acid Buffer (see below)
- *Vortex to mix*
- *Prepare fresh for each use*

Eosin Y solution aqueous **Merck Catalog #**HT110216-500ML

Tris-Acetic Acid Buffer: 200mL

- Dissolve 11g Tris base in 100mL nuclease-free water (Fisher, BP152-500)
- Adjust pH to 6.0 using 100% Acetic Acid (Fisher, A38-212)
- Bring volume to 200mL with nuclease-free water
- *Filter through 0.2µm Corning 250mL Vacuum System*
- *Store at room temperature for up to 12 months*

Tris Base **Fisher Scientific Catalog #**BP152

Acetic acid Glacial **Fisher Scientific Catalog #**A38-212

Nuclease-free Water

Protocol:

- Tissue slice from OCT embedded tissue block, mounted on a 10X Visium slide
- Methanol suitable for HPLC ≥99.9% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860
- Mayer's Hematoxylin **Dako Catalog #**S3309
- Shandon™ Bluing Reagent **Thermo Fisher Catalog #**6769001
- Eosin Y solution aqueous **Merck Catalog #**HT110216-500ML
- Tris-Acetic Acid Buffer (see above)
- Eosin Mix (see above)
- Nuclease-free water or water filtered using a Milli-Q filtering system **Ambion Catalog #**AM9932
- 10X Visium Thermocycler Adaptor (part of 10X Genomics Visium Accessory Kit; 1000194)

Visium Accessory Kit **10x Genomics Catalog #**1000194

Equipment

Thermal cycler	NAME
T100 PCR thermal cycler	TYPE
BIO-RAD	BRAND
https://www.bio-rad.com/pt-br/product/t100-thermal	SKU
https://www.bio-rad.com/pt-br/product/t100-thermal-cycler?ID=LGTWGIE8Z	LINK

Troubleshooting

Before start

Prepare Eosin mix fresh for each use. The tris-acetic acid buffer can be stored for up to 12 months.

Tissue Fixation

1 Prechill methanol (40mL/slide, dispensed in a 50-mL centrifuge tube) to -20 °C .

2 Place a Thermocycler Adaptor on a thermal cycler set at 37 °C and equilibrate for 00:05:00 . Heating the Thermocycler lid is not required. 5m

3 Remove slide from -80°C and place on dry ice in a sealed container.

4 Place slide on the Thermocycler Adaptor with the active surface facing up and incubate 00:01:00 at 37 °C . 1m

4.1 DO NOT close the thermocycler lid. Maintain thermal cycler at 37 °C .

5 Remove slide from Thermocycler Adaptor and if necessary, wipe excess liquid from the back of the slide, without touching the tissue sections.

6 Completely immerse the slide in the prechilled -20 °C methanol.

6.1 Secure the tube cap to prevent methanol loss.

7 Incubate upright for 00:30:00 at -20 °C . 30m

Tissue H&E Staining 19m

8 Dispense the following volumes of Milli-Q water:
a. 500mL in Beaker 1
b. 800mL in Beaker 2
c. 800mL in Beaker 3
d. 800mL in Beaker 4

NOTE: Dispensed volume in each beaker can be used for two slides.

9 Prepare Eosin Mix.

9.1 DO NOT add pure eosin to tissue sections.

10 Remove slide from methanol and wipe excess liquid from the back of the slide, without touching the tissue sections.

11 Place on a flat, clean, nonabsorbent work surface.

Note: Some residual droplets may remain.

12 Add 500µL isopropanol to uniformly cover all tissue sections on the slide.

13 Incubate 00:01:00 at room temperature. 1m

Tip: When incubating the slide with reagents, ensure that the slide is not in contact with any absorbent surface, like laboratory wipes, which may absorb the reagents.

14 Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

15 Wipe excess liquid from the back of the slide, without touching the tissue sections.

16 Place on a flat, clean, nonabsorbent work surface.

Note: Some droplets may remain.

17 Air dry the slide for 00:04:00 . 4m

18 Add 1mL Hematoxylin to uniformly cover all tissue sections on the slide.

19 Incubate 00:05:00 at room temperature. 5m

20 Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

21 Immerse the slide 5x in the water in Beaker 1.

22 Immerse the slide 15x in the water in Beaker 2.

23 Immerse the slide 15x in the water in Beaker 3.

24 Wipe excess liquid from the back of the slide without touching the tissue section.

25 Place on a flat, clean, nonabsorbent work surface.

Note: Some droplets may remain.

26 Add 1mL Bluing Buffer to uniformly cover all tissue sections.

27 Incubate 00:02:00 at room temperature. 2m

28 Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe

29 Immerse the slide 5x in the water in Beaker 3.

30 Wipe excess liquid from the back of the slide without touching the tissue section.

31 Place on a flat, clean, nonabsorbent work surface.

Note: Some droplets may remain.

32 Add 1mL Eosin Mix to uniformly cover all tissue sections.

33 Incubate 00:02:00 at room temperature. 2m

34 Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

35 Immerse the slide 15x in the water in Beaker 4.

36 Wipe the back of the slide with a laboratory wipe.

37 Place on a flat, clean, nonabsorbent work surface and air dry until tissue is opaque.

38 Incubate slide on the Thermocycler Adaptor with the thermal cycler lid open for 00:05:00 at 37 °C . 5m

39 Proceed to tissue imaging.

Note: Ensure that the entirety of the tissue slice is in the same focal plane before imaging, to reduce the risk of stitching-induced image artifacts hindering downstream analyses.