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H&E Staining for Pancreas or Eye Cryosections V.2

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VU Biomolecular Multimodal Imaging Center

Human BioMolecular Atlas Program (HuBMAP) Method Development Community



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This protocol for H&E staining can be applied to either fixed or unfixed frozen cryosections.

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HuBMAP, BIOMIC, Vanderbilt, Pancreas, H&E staining, Eye, Retina

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1. Coplin jars
2. 95% Ethanol
3. Hematoxylin
4. Bluing Solution
5. Eosin
6. 100% Ethanol
7. Xylene
8. Coverslips
9. Cytoseal

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions.

2. Xylenes should be used in the fume hood.

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|---|--|-----|
| 1 | Air dry sections for 🕒00:05:00 . | 5m |
| 2 | Incubate in 95% alcohol for 🕒00:01:00 , then wash, dipping until clear. | 1m |
| 3 | Incubate in hematoxylin for 🕒00:00:30 , then wash until clear. | 30s |
| 4 | Dip 3-4x in bluing solution , then wash for 🕒00:00:30 | 30s |
| 5 | Dip 3-4x in 95% alcohol . | |
| 6 | Dip 1-2x in eosin . | |
| 7 | Dip 5-10x in 95% alcohol , then repeat using 2nd aliquot. | |

- 8 Dip 5-10x in **100% alcohol**, then repeat using 2nd aliquot.
- 9 Dip 5-10x in **xylene**, then repeat using 2nd aliquot.
- 10 Apply coverslip and seal.

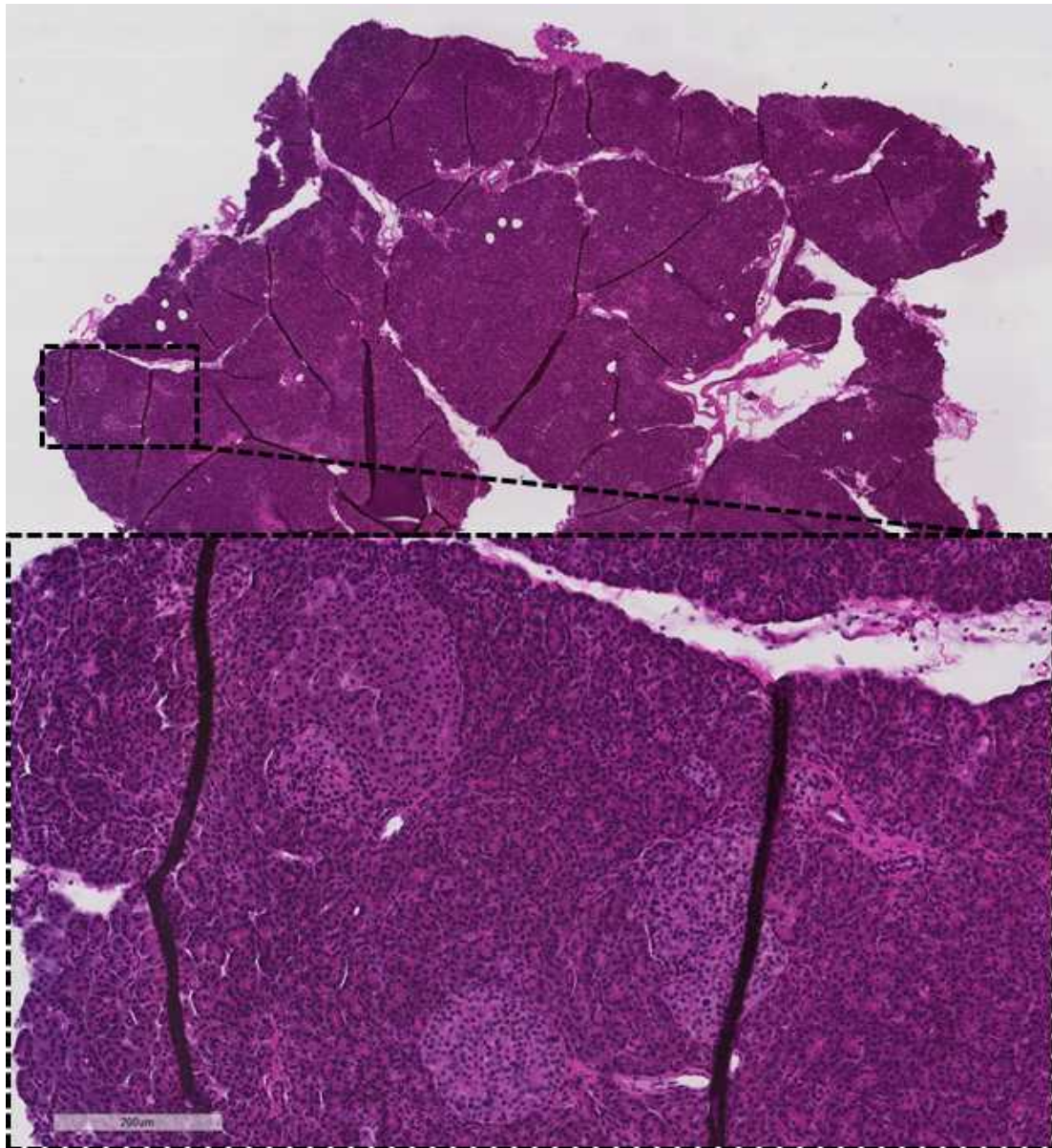


Figure 1. H&E stained pancreas with magnified section showing nuclei in blue and cytoplasm in pink.



Figure 2. H&E stain of human retina tissue from a 72 year old donor. Dark purple nuclear staining and pink cytoplasmic staining allow the user to readily distinguish retinal layers such as (from bottom to top) the: sclera, choroid, retinal pigmented epithelium, photoreceptor layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, and nerve fiber layer.