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Post-IMS H&E Staining for Pancreas or Eye Cryosections

H&E Staining for Pancreas or Eye Cryosections

Angela Kruse¹, Diane Saunders¹, Jamie Allen¹, Carrie Romer¹,
Danielle Gutierrez¹, Alvin Powers¹, Jeff Spraggins¹

¹Vanderbilt University

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protocol .

VU Biomolecular Multimodal Imaging Center

Tech. support email: jeff.spraggins@vanderbilt.edu

Angela Kruse
Vanderbilt University

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

Angela Kruse, Diane Saunders, Jamie Allen, Carrie Romer, Danielle Gutierrez, Alvin Powers, Jeff Spraggins 2022. Post-IMS H&E Staining for Pancreas or Eye Cryosections. **protocols.io**
<https://protocols.io/view/post-ims-h-e-staining-for-pancreas-or-eye-cryosect-b9ywr7xe>



This protocol is specifically for H&E staining after IMS data acquisition.

H&E Staining for Pancreas or Eye Cryosections, Carrie Romer

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.

Matrix Removal 7m

- 1 Collect [post-IMS autofluorescence](#)
- 2 Remove matrix from tissue sections by incubating in **100% alcohol**, dipping gently for  **00:00:30** . Repeat this step 2-3 times until there is no visible matrix on the slides. 30s

H&E Staining 7m

- 3 Incubate in **95% alcohol** for  **00:01:00** , then wash, dipping until clear. 1m
- 4 Incubate in **hematoxylin** for  **00:00:30** , then wash until clear. 30s
- 5 Dip 3-4x in **bluing solution**, then wash for  **00:00:30** 30s
- 6 Dip 3-4x in **95% alcohol**.
- 7 Dip 1-2x in **eosin**.

- 8 Dip 5-10x in **95% alcohol**, then repeat using 2nd aliquot.
- 9 Dip 5-10x in **100% alcohol**, then repeat using 2nd aliquot.
- 10 Dip 5-10x in **xylene**, then repeat using 2nd aliquot.
- 11 Apply coverslip and seal.

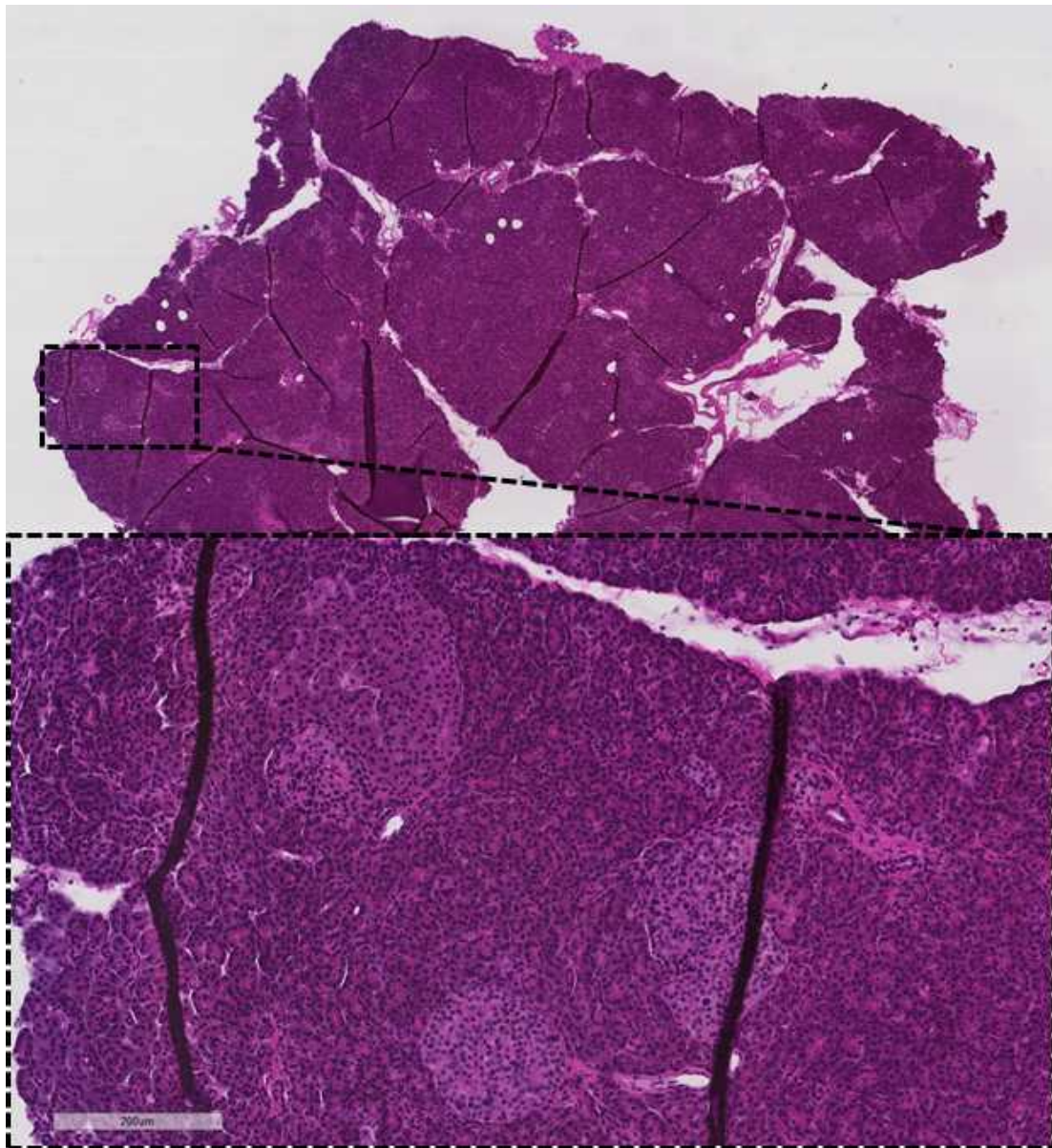


Figure 1. H&E stained pancreas with magnified section showing nuclei in blue and cytoplasm in pink.



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.