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

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Protocol status: Working

We use this protocol and it's working

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Keywords: HuBMAP, BIOMIC, Vanderbilt, Pancreas, H&E staining, Eye, Retina, eye cryosection, unfixed frozen cryosection, eye cryosections this protocol, staining

Abstract

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

Materials

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

Safety warnings

- ! 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.
- 4 Incubate in **hematoxylin** for  00:00:30 , then wash until clear.
- 5 Dip 3-4x in **bluing solution**, then wash for  00:00:30
- 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.

1m

30s

30s

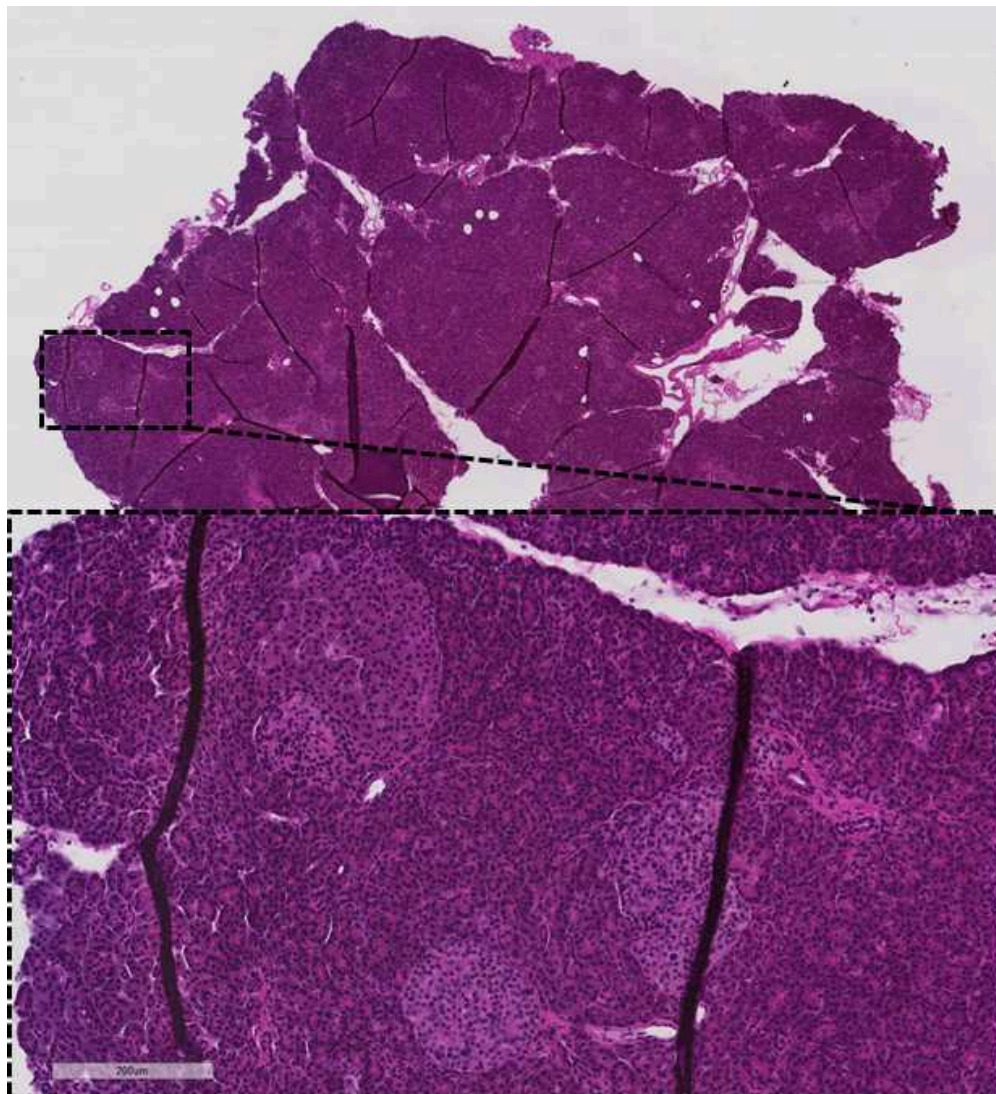


Figure 1. H&E stained pancreas with magnified section showing nuclei in blue and cytoplasm in pink.



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.