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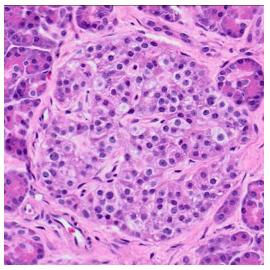
Hematoxylin and Eosin (H&E) Staining for Human Pancreas



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We use this protocol and it's working

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Abstract

PURPOSE

To provide instructions for using the automated Leica AutoStainer XL instrument to produce quality hematoxylin and eosin (H&E) stained slides from fixed paraffin-embedded samples for human pancreas studies by the HubMap Tissue Mapping Center- PNNL/UF (U54DK127823 (Qian, contact PI).

SCOPE

This procedure applies to tissue sections to be stained with H&E and includes instructions for properly using the Leica AutoStainer XL. This protocol is also used for paraffin-embedded sections following assay on the Nanostring GeoMx digital spatial profiler instrument and large paraffin-embedded sections placed on large format slides (2" x 3" slides).

RESPONSIBILITIES

The Lab Manager, or designate, is responsible for making sure that staff members are properly trained and equipment is appropriately cleaned, maintained in good working order, and available as requested.

Lab staff members are responsible for reading and understanding this SOP and related documents and performing these tasks in accordance with the SOPs including cleaning and solution rotations and/or changes.

Attachments

PDF

SOP 421 Slide Staini...

1,006KB

Image Attribution

HubMap human pancreatic islet (P6-19B), 4% paraformaldehyde-fixed paraffin-embedded, 4 µm thick section stained by H&E according to protocol.

Materials

1.

Equipment	
Autostainer XL	NAME
Histology	TYPE
Leica	BRAND
8226-30-0006	SKU
https://www.leicabiosystems.com/us/histology-equipment/he-slide-stainers-special-stainers-coverslippers/leica-st5010/	LINK

2. Supplies
Leica Staining racks and rack holders- multiple
Rack adaptor for staining supa mega slides- Electron Microscopy Services 70065-30
Whatman #1 filter paper, 32 cm diameter- Sigma WHA1001320
Glass funnel and 500ml glass beaker

3. QC slide
Quality control FFPE H&E slide containing representative tissues from human.

4. Reagents
Xylene **Fisher Scientific Catalog #**X3P-1GAL
Ethanols, 95%, 100%- Fisher Scientific 04-355-226, 04-355-223
Hematoxylin 7211- Fisher Scientific, 22-050-111
Clarifier 1- Fisher Scientific for Richard Allan, 22-050-118
Bluing Reagent- Fisher Scientific, 22-050-115
Eosin-Y alcoholic- Fisher Scientific for Richard Allan, 22-110-637

Suitable alternative reagents can be used at management's discretion.

Troubleshooting

Safety warnings

All reagents should be handled with the proper PPE.
Follow universal safety precautions when staining unfixed human samples (e.g., face mask with shield, gloves, lab coat or apron).
Operate equipment with suitable air exhaust or within a fume hood.
For detailed information, consult the MSDS website for each chemical.
1.1 Xylene: Prolonged or repeated skin contact can cause moderate irritation and defatting dermatitis. Avoid eye contact. Keep xylene containers in the autostainer covered with lids when not in use.
1.2 Ethanol: Harmful vapors, use adequate ventilation. Avoid contact with skin, eyes, and clothing.
1.3 Hematoxylin 7211: Harmful vapors, use adequate ventilation. Avoid contact with skin, eyes, and clothing. Fatal or harmful if swallowed.
1.4 Eosin Y: Highly flammable. Harmful vapors, use adequate ventilation. Avoid contact with skin, eyes, and clothing. Ingestion may cause poisoning.
1.5 Clarifier 1: Contains acetic acid. Avoid inhalation, ingestion or skin contact. If contact occurs, flush affected area with water.

Operation

1 Filter, change and/or rotate solutions according to **Attachment** page 4. Filter Hematoxylin before first use each day.

2 An approved control fixed paraffin-embedded slide containing human pancreas, brain, liver, and/or kidney is stained after solution changes/substitutions or equipment maintenance to verify stain quality.

2.1 The designee will be responsible for determining staining quality for the control slide.

2.2 Label control slide with stain and date and store stained control slides for as long as required. Use to validate new reagents and times.

3 Select the desired program by pushing **Stain** key (F1) and use up/down arrows on keypad to select desired program (see **Attachment** page 5).

4 Load dried slides, labeled end up, in a rack. Place rack in the autostainer via the load drawer at the front right hand side. To open the drawer, grasp and push up on the release lever on the underside of the drawer and pull outward.

5 Press **Load** key. Slides will be stained according to the selected program.

	Station	TIME	Maintenance	Purpose
1. Oven		15 min		Melt paraffin wax
2. Xylene	1	3 min	Rotate every 200	Deparaffinization
3. Xylene	2	3 min		Deparaffinization
4. Xylene	3	3 min		Deparaffinization
5. 100% EtOH	4	1 min	Rotate every 200	Xylene clearing
6. 100% EtOH	5	1 min		Xylene clearing
7. 95% EtOH	6	1 min	Rotate every 200	Rehydration
8. 95% EtOH	7	1 min		Rehydration
9. Water	Wash 1	1 min		Rehydration
10. Hematoxylin 7211	8	3 min	Filter day of use	Nuclear stain
11. Water	Wash 2	3 min		Remove excess
12. Clarifier 1	9	45 sec	Change every 200	Differentiation
13. Water	Wash 3	1 min		Remove clarifier
14. Bluing Reagent	10	1 min	Change every 200	Convert Hemalum to blue color
15. Water	Wash 4	1 min		Return pH to neutral
16. 95% EtOH	11	30 sec	Change every 200	Dehydration
17. Eosin-Y	12	45 sec		Cytoplasmic stain
18. 100% EtOH	13	1 min	Rotate every 200	Remove excess Eosin
19. 100% EtOH	14	1 min		Dehydration
20. 100% EtOH	15	1 min		Dehydration
21. Xylene	16	1 min	Rotate every 200	Clearing
22. Xylene	17	1 min		Clearing
23. Xylene	Exit	Hold		Clearing
End program				

Representative station times by reagent, station, time, maintenance procedure, and purpose. From **Attachment** page 5.

6 When a rack is in the exit station, the (Exit) LED will be on and the beeper will sound every 30 seconds. To unload the rack, open the exit drawer and remove the rack.

6.1 Remove the entire xylene reagent container or transfer slide rack to another xylene reagent container of xylene and move to fume hood for coverslipping.

7 Record the number of slides stained on the H&E Staining Log. Place a checkmark in each column to indicate whether that solution was rotated, changed, filtered, or topped off. Enter the number of
slides stained during the day in the “Number of Slides” column and place the cumulative total number of slides in the “Total Slides Stained” column. This number will be reset to zero after ~200 slides have been stained.

Maintenance and Cleaning

8 The Leica autostainer must be properly installed and maintained to achieve optimum results. The basic operation of the stainer will be in accordance with the operating manual furnished by the manufacturer.

8.1 Clean reagent containers using soap and water then dry before use. Use bleach to remove hematoxylin staining.

8.2 Change the activated carbon filter annually or as indicated by fume hood manufacturer.

8.3 Record maintenance on the Equipment Repair Record and perform annual preventative maintenance by trained service technicians.

8.4 In the event the Leica autostainer is unavailable, slides can be stained manually in appropriate containers using the same reagents and times.

Expected Results

9 Pancreas: Nuclei of cells within islets should stain purple with darker nucleoli. Nuclei of acinar cells stain purple. Cytoplasm should stain pink except for blue acinar cell zymogen granules. Collagen, RBCs, smooth muscle, and cytoplasm should be easily differentiated in the exocrine pancreas.

10 Brain: Cytoplasm should stain pink. If it is gray or blue, there is too much hematoxylin. Cerebellar Purkinje cells should be purple.

11 Liver: Nuclei of cells in the hepatic triads are dense and the hepatocytes have an open euchromatin pattern. Collagen, RBCs, smooth muscle, and cytoplasm should be easily differentiated in the hepatic triads.

12 Kidney: Nuclei of cells within the glomerulus have dense chromatin. The proximal tubules have open euchromatin. The large blood vessels should demonstrate clear differentiation of RBCs, collagen, smooth muscle, and cytoplasm.

Protocol references

Campbell-Thompson ML, Heiple T, Montgomery E, Zhang L, Schneider L. Staining protocols for human pancreatic islets. Journal Visual Experimentation, 2012 May 23;(63). pii: 4068. doi:10.3791/4068. PMID: **22665223**

H&E Staining Overview: A Guide to Best Practices **link**

Acknowledgements

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