Oct 18, 2022

H&E

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqj79kvk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.36wgqj79kvk5/v1

Protocol Citation: emonte 2022. H&E. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqj79kvk5/v1

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

Created: October 18, 2022

Last Modified: October 18, 2022

Protocol Integer ID: 71466

Keywords: amp

Abstract

H&E

1 H&E staining protocol for pathology lab is here.

Stanford MEDICINE Health Care		**Last Approval Date:** 09/21/2022
Title: Tissue-Tek Prisma Automated Slide Stainer		**Approved by:** Neeraja Kambham
Departments Affected: Histology Services		Page 1 of 5
Key Words: Histology: 012	**Reference #:** 6410	

I. PURPOSE:

A. The use of automation to stain H&E's can produce consistent, quality results. The Tissue-Tek Prisma Autostainer is an automatic staining system which can be programmed to carry out multiple staining protocols simultaneously.

II. APPLICABILITY:

A. This policy applies to all Histotechnologists, Histotechnicians and Lab Techncians working in the Histology laboratory.

III. ASSOCIATED DOCUMENTS:

A. Tissue-Tek Prisma Automated Coverslipper and Manual Coverslipping Procedure
B. Maintenance for Prisma Autostainer and Coverslipper (Form-038)
C. Daily QC by Histology (Form-036)
D. Equipment Quality Control Policies and Procedures
E. H&E Staining Solution Lot#'s Log (Form-041)
F. Prisma H&E Stain Set Up (Form-128)

IV. DEFINITIONS:

A. Control and monitoring screen – A color touch screen display where all operations are controlled.
B. Solution reservoir – Container within the Prisma stainer holding reagents to be used in the staining protocol.
C. Link System – Used to link the instrument with the Tissue-Tek Film coverslipper.

V. EQUIPEMENT / REAGENTS / CONSUMABLES:

A. Xylene- Cardinal Health- Cat. # C4330, or equivalent
B. 100% Dehydrant –Cardinal Health-Cat. # C4305-10, or equivalent
C. 95% Dehydrant-Cardinal Health-Cat. # C4305-12, or equivalent
D. Hematoxylin-Richard Allen Scientific-Cat. # 7211
E. Eosin Y- Richard Allen Scientific Cat. # 7111
F. Clarifier- Richard Allen Scientific Cat. #7401
G. Bluing Reagent-Richard Allen Scientific Cat. #7301
H. Tissue-Tek Coverslipping film- Sakura Finetek Cat. # 4770

VI. SPECIMEN SUBMISSION REQUIREMENTS:

A. Slides made from paraffin tissue blocks

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VII. CONTROLS:

A. Daily quality control checks of the H&E stain are performed by a Histotechnologist/Histotechnician and the resident at the Hot Seat. This is documented on Form-036 by HT and on a digital excel file in Box by Hot Seat..

B. Reagent lot to lot verification is recorded on the H&E Staining Solution Lot#'s log (Histo Form-041).

C. Documentation and frequency of changing solutions on the Prisma Autostainer is recorded on Maintenance for Prisma Autostainer and Coverslipper (Form-038).

VIII. PROCEDURES:

A. Stain maintenance is conducted as follows (using Form-128 as a guide to station numbers and Form-038 to document):

1. Daily Maintenance
   a. **All alcohol** solutions changed.
   b. Discard clarifier and bluing, replace with fresh solutions.
   c. De-paraffin xylenes: first and second dumped, third rotated to first position, second and third replenished with fresh xylene.
   d. End xylenes; first xylene dumped, second and third rotated to first and second position, third replenished with fresh xylene.
2. **Every other day – (Sunday, Tuesday, Thursday)**
   a. **All solutions** are changed on stainers.
   b. Discard and replace hematoxylin and eosin with fresh solutions.
      i. The dirty eosin container is rinsed thoroughly before replenished with fresh eosin.
      ii. The dirty hematoxylin container is soaked in bleach solution overnight then put through dishwasher for cleaning and use on next change.

B. Turn **ON** power switch located in lower right-hand corner of the unit. Control monitor screen will appear.

C. Touch **START** icon on the monitor screen.

D. Open stainer access door and hang basket(s) containing slides on the staining arm hook. Before loading slides onto the stainer, make sure specimen side of the slide must face forward towards words UP SIDE on the basket and the labeled end of the slide is to the outside (top of the basket). Attach basket holders with thin end of the handle towards words UP SIDE.

E. Close stainer access door and touch **START** icon on the monitor. Stainer will begin.

1. STANFORD H&E PROTOCOL
   a. Xylenes- 30 sec, 1 min and 1 min
   b. 100% Alcohol-30 sec and 1 min

c. 95% Alcohol- 30 sec each
d. Wash station- 1 min.
e. Hematoxylin- 4 min.
f. Wash Station- 1 min
g. Clarifier- 40 sec.
h. Wash Station- 1 min
i. Bluing- 30 sec.
j. Wash Station- 1:30 min
k. 95% Alcohlol- 30 sec
l. Eosin Y- 1 min
m. 95% Alcohol- 30 sec
n. 100% Alcohol- 1 min each
o. Xylenes- 30 sec, 30 sec and 1 min

F. When staining is complete, slide baskets will automatically link to the automated coverslipper.
G. If "link" is disabled, coverslip following "Tissue-Tek Prisma Automated Coverslipper and Manual Coverslipping Procedure"

IX. CONTINGENCIES:

A. If for any reason this operating procedure cannot be followed as written, the Histology Supervisor must be informed immediately. Alternative processes must be documented, reviewed and approved by the Histology supervisor and the Operations manager.

X. RESPONSIBILITIES:

A. It is the responsibility of Histology staff members to follow the above protocol as outlined.
B. It is the responsibility of the Histology Supervisor or designee to review all QC and maintenance logs.

XI. MISCELLANEOUS:

A. Appropriate personal protective equipment should be worn while handling specimens which should at least include gloves and lab coats.
B. Document all maintenance activities on the appropriate logs.
C. Patient specimens must be considered infectious and standard safety precautions followed.

XII. REFERENCES:

A. For a more detailed reference, please refer to "Tissue-Tek Prisma Plus Automated Slide Stainer and Tissue-Tek Film Automated Coverslipper" operating manuals.

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XIII. COMPLIANCE:

A. All workforce members including employees, contracted staff, students, volunteers, credentialed medical staff, and individuals representing or engaging in the practice at Stanford Health Care (SHC) are responsible for ensuring that individuals comply with this procedure.

B. Violations of this procedure will be reported to the Department Manager and any other appropriate department as determined by the Department Manager or in accordance with SHC policy. Violations will be investigated to determine the nature, extent, and potential risk to SHC. Workforce members who violate this procedure will be subject to the appropriate disciplinary action up to and including termination.

XIV. DOCUMENT INFORMATION:

A. Legal References / Regulatory Requirements:
   1. ANP.21360

B. Original Document:
   1. E. Sabato August 2008
   2. Stored in: AP Histo

C. Review and Renewal Requirements:
   1. This procedure will be reviewed and/or revised every two years or as required by change of law or practice.

D. Review and Revision History:
   1. D. Burns and P. Bhavsar September 2020
   2. P. Bhavsar and D. Burns August 2022

E. Approvals:
   1. Gerald Berry, MD September 2020
   2. N. Kambham, MD September 2022

Change Summary

Version	Description of Change
v.2	Reviewed the document, updated the H&E procedure steps and updated using new format.
v.3	Document reviewed and updated in title for medical director, Form 37 removed from

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