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We use this protocol and it's working

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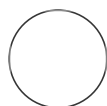
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U54 SCENT Histology H&E Staining V.1

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Cellular Senescence Network (SenNet) Method Development Community



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ABSTRACT

To provide a standardized method of running, cleaning, refilling, and verifying quality of the automatic H&E stainer. Every morning before running slides for projects, a quality control slide containing brain, kidney, liver, and pancreas tissue is run, reviewed, and deemed acceptable by a histotechnician, who documents this review.

MATERIALS

Personal Protective Equipment:

- ☐ Lab coat
- ☐ Gloves

Equipment:

- ☐ Leica Autostainer XL
- ☐ Microscope

Materials:

- ☐ Bleach
- ☐ Xylene
- ☐ Alcohol, 100%
- ☐ Alcohol, 95%
- ☐ Alcohol, 80%
- ☐ Hematoxylin –Pureview Hematoxylin 660+
- ☐ Eosin – Pureview Eosin 615
- ☐ Clearing Solution –Pureview Focus-AQ
- ☐ Bluing – Pureview PH Blue
- ☐ Chemical waste container
- ☐ Wypall pads
- ☐ Slide staining rack
- ☐ Pre-cut H&E control slide

Protocol - Operating Autostainer and Running Daily Quality...

- 1 Open cover of autostainer and remove each lid of the reagents inside. Stack lids and set to the right side of autostainer. Close cover.
- 2 Hit switch on the right side of autostainer to turn on and initialize.
- 3 Once initialization is complete, press button on screen to select staining program 1.
- 4 Take one slide from the box of unstained H&E control slides and label with:
 - a. H&E QA
 - b. Date
 - c. Tech initials
- 5 Place the slide into a black 30-count slide rack.
- 6 Pull the lever below the loading drawer on the autostainer to open.
- 7 Place slide rack inside and close.
- 8 Hit button to the left of loading drawer to begin staining run.
- 9 When staining has finished, autostainer will periodically beep.

- 10 Pull the lever below the exit drawer on the autostainer to open.
- 11 Press button next to exit drawer so instrument knows the rack was removed. (Autostainer will continue to beep otherwise.)
- 12 Coverslip and microscopically verify correct staining of control tissues.
- 13 Denote on RHL Internal H&E Histologic Quality Check. If staining quality is suboptimal, troubleshoot.
- 14 If staining is acceptable, write "PASS" on slide and store in labeled control slide box next to autostainer.
- 15 File form in H&E quality control binder at the end of the month.
- 16 Any research slides for the day can be loaded in the same way once the review is complete.
- 17 At the end of the workday, open cover and replace reagent lids to prevent evaporation. Close cover.

Station #	Solution	Time
1	Xylene	2 min
2	Xylene	2 min
3	Xylene	2 min
4	100% Alcohol	2 min
5	100% Alcohol	30 sec
6	95% Alcohol	2 min*
7	95% Alcohol	1 min
Wash 5	Water wash	1 min
8	Hematoxylin*	30 sec*
Wash 4	Water Wash	30 sec
Wash 3	Water Wash	2 min
9	Clearing Solution	30 sec
Wash 2	Water Wash	1 min
10	Bluing Solution	30 sec
Wash 1	Water Wash	30 sec
11	80% Alcohol	30 sec
12	Eosin*	30 sec
13	95% Alcohol	30 sec
14	95% Alcohol	30 sec
15	100% Alcohol	30 sec
16	100% Alcohol	30 sec
17	Xylene	30 sec
18	Xylene	30 sec
Exit (19)	Xylene	Hold-exit station

*These are the only two stations with hard times. Slides may be held longer in other stations when multiple racks are being run at the same time.

Protocol - Changing the Autostainer

containers located below autostainer

- 20** Rinse containers out with water and, working over the sink, fill with a 10% bleach solution for at least 15 minutes or until visibly clean.
- 21** Rinse out containers and place upright on a counter that has been covered with Wypall pads. Leave to air dry over the weekend.
- 22** Fill water wash containers with 10% bleach and then 3 changes of water to remove bleach residue (containers will automatically drain when filled).
- 23** Flush 10% bleach solution followed with 3 changes of water through the base of the stainer. Use a bleach-soaked Wypall pad to spot clean any splashed staining reagents. NOTE: Manual H&E stain line should remain set up until autostainer is refilled and tested the next week to ensure one staining line is always available for use.
- 24** At the beginning of the work week, refill staining containers and place back into autostainer.
- 25** Reagent setup is as follows:

A	B	C	D	E	F	G	H	I	J	K	L
Xylene	Xylene	Xylene	100% Alcohol	100% Alcohol	95% Alcohol	95% Alcohol	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5
Xylene	Xylene	Xylene	100% Alcohol	100% Alcohol	95% Alcohol	95% Alcohol	Eosin	80% Alcohol	Bluing Solution	Clearing Solution	Harris Hematoxylin

- 26** Mark reagents changed on Autostainer Maintenance Log.

