



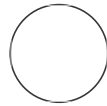
VERSION 1

JAN 16, 2024

Brain Histology - tissue sectioning and staining V.1

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ABSTRACT

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Protocol Citation: Yiqin Shen 2024. Brain Histology - tissue sectioning and staining. **protocols.io** <https://protocols.io/view/brain-histology-tissue-sectioning-and-staining-c7ntzmen>

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

Created: Jan 16, 2024

Last Modified: Jan 16, 2024

PROTOCOL integer ID:
93619




Sectioning




- 1 Turn on and set the water floating device to  37 °C .
Prepare a bucket of ice. Place the samples on ice before sectioning.
Check the blades by doing a few test sections. Change if they are not sharp.
- 2 Place a block of sample on the holder.
Adjust the machine so the block is near the blade but not cut. Rotate the handle to bring near the block.
- 3 Start sectioning. Avoid ruptures and curling.

Note

If the samples are not cutting well, either check the blade, or place the sample back on ice for a few minutes depending on the situation.
- 4 Place the slice on to water with brush, and gently catch them with glass slides. Label each slide well.
Allow the slides to air dry.






Staining- Day 1


- 5 Place the slides onto the slide holder, and dip in xylene for  00:30:00 . 30m
- 6 Wash the slides with alcohol. The washes are at different concentrations from 100% to 50%,
 00:06:00 each. 6m
Rinse with DD H2O
- 7 Remove the slides from holder, trace the outside with hydrophobic pen.
- 8 Add blocking buffer (Bloxall) to the slides for  00:30:00 . 30m
Rinse with PBS 2X 3min

- 9 Place the slides onto the holder. It should be cooked with pressure cooker in citrate buffer for  00:25:00 . 25m
- 10 Remove the slides from the holder and place them down.
Add 2.5% horse serum to the slides for  00:25:00 .
Wash with PBS 2X 3 min. 25m
- 11 Dilute primary antibody in blocking buffer, add to the slides.
Incubate in the fridge  Overnight 4C 25m

Staining- Day 2

2h 21m

- 12 Rinse the slides with PBS.
Dip in TBS-T for  00:10:00 for three times.
Rinse with PBS. 10m
- 13 Add blocking buffer to the slides for  01:00:00 1h
- 14 Add secondary antibody to the slides, at  Room temperature  01:00:00 . 1h
- 15 Rinse the slides with PBS.
Dip in TBS-T for  00:10:00 for three times.
Rinse with PBS. 10m
- 16 Develop color with chromogen, rinse in DD H2O to stop reaction

- 17 Put the slides in Hematoxylin for 30s
Rinse 2X DD H2O, and allow the slides to air-dry.
- 18 Put the slides in Xylene for  00:01:00 two times.
- 19 Coverslip + mounting media

1m