Brain Histology - tissue sectioning and staining V.1

Yiqin
Shen[1]

[1]University of California, San Diego

Yiqin Shen
University of California, San Diego

VERSION 1
JAN 16, 2024

OPEN ACCESS

ABSTRACT

.

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

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%, 6m
00:06:00 each.
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. 25m
Add 2.5% horse serum to the slides for 00:25:00 .
Wash with PBS 2X 3 min.

11 Dilute primary antibody in blocking buffer, add to the slides. 25m
Incubate in the fridge Overnight 4C

Staining- Day 2

2h 21m

12 Rinse the slides with PBS. 10m
Dip in TBS-T for 00:10:00 for three times.
Rinse with PBS.

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. 10m
Dip in TBS-T for 00:10:00 for three times.
Rinse with PBS.

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. 1m

19 Coverslip + mounting media