




Sep 14, 2022

# Nissl Staining

Pranay Srivastava<sup>1</sup>, Xiqun Chen<sup>1</sup>

<sup>1</sup>MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, USA Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Towson, MD, USA

1 Works for me

 Share[dx.doi.org/10.17504/protocols.io.bp2l6bz9zgqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l6bz9zgqe/v1) ktdonahue

## ABSTRACT

This protocol details the staining procedure and mounting procedure of Nissl staining.

## DOI

[dx.doi.org/10.17504/protocols.io.bp2l6bz9zgqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l6bz9zgqe/v1)

## PROTOCOL CITATION

Pranay Srivastava, Xiqun Chen 2022. Nissl Staining. **protocols.io**  
<https://protocols.io/view/nissl-staining-bvvgn63w>



## KEYWORDS

Nissl Staining, Staining Procedure, Mounting Procedure, ASAPCRN

## LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

Jun 16, 2021

## LAST MODIFIED

Sep 14, 2022

## OWNERSHIP HISTORY

Jun 16, 2021  Urmilas

Jun 21, 2021  ktdonahue

### Solutions and Reagents:

0.1% Cresyl violet solution:

A	B
Cresyl echt violet (or cresyl violet acetate)	0.1g
Distilled water	100 ml


Add 10 drops (0.3 ml) of glacial acetic acid just before use and filter.

## Staining Procedure

1h 19m

1 

Mount sections on positive charged plus slides. Air dry sections  **Overnight** .

2 Incubate the slides directly into 1:1 ethanol/chloroform for  **00:30:00** . 30m  
(All the steps mentioned should be done under the hood)

3 Incubate in 100% ethanol  **00:02:00** 2m

4 Incubate in 95% Ethanol  **00:02:00** 2m

5 Incubate in 70% Ethanol  **00:02:00** 2m

6 Incubate in 50% Ethanol  **00:02:00** 2m

7 Wash in distilled water for  **00:05:00** 5m

8 Stain in 0.1% cresyl violet solution for ⌚00:06:00 .

6m

Notes: staining in warmed cresyl violet solution (warm up to ⌆ 37 °C - ⌆ 50 °C ) can improve penetration and enhance even staining. It is particularly beneficial for thicker ( 📏30 undetermined ) sections.

9 

Rinse quickly in distilled water.  
(check the slides for the best result under the microscope)

10 Incubate in 50% ethanol (3 dips in the container)

10.1 Incubate in 95% ethanol (3 dips in the container)

11 Incubate in 100% ethanol (3 dips in the container)

12 Incubate in xylene ⌚00:03:00

3m

### Mounting Procedure

13 

Pour ~ 📏3 mL Permount solution into a 📏5 mL tube and use a transfer pipette for mounting.

14 Remove one of the slides from the xylene with tweezer and dry by dabbing it on a paper towel. Then place down flat.

15 Put 6-7 drops of Permunt along the middle of the slide.

16 Take a cover slip and place the left side down onto the slide.

You want to gently and slowly lower down the right side of the cover slip so that you minimize air bubbles in between the cover slip and the slide. To do so, use a needle to hold the right side, while using the needle's cap to hold the left side down in place.

17 Once the cover slip is completely on the slide, drain any extra permunt on a paper towel and then lay the slides to dry.

18 If you make a mistake while cover slipping, put the slide with the cover slip back into xylene.

This will remove the permunt and the cover slip, allowing you to try again.