

Jan 08, 2026

Immunolabelling and clearing of intact, fixed rat spinal cord for visualization of motor nuclei

 Forked from [Immunolabelling and clearing of intact spinal cord for visualization of lower urinary tract afferents](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwjq47lmk/v1>

John-Paul Fuller-Jackson¹, Peregrine B Osborne¹, Janet R Keast¹

¹University of Melbourne

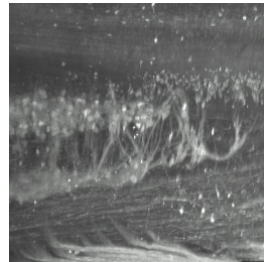
SPARC

Tech. support email: info@neuinfo.org



Janet R Keast

University of Melbourne



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwjq47lmk/v1>

Protocol Citation: John-Paul Fuller-Jackson, Peregrine B Osborne, Janet R Keast 2026. Immunolabelling and clearing of intact, fixed rat spinal cord for visualization of motor nuclei. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwjq47lmk/v1>



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 06, 2023

Last Modified: January 08, 2026

Protocol Integer ID: 78162

Keywords: neural tracing, retrograde tracing, clearing, idisco, light sheet microscopy, rat spinal cord for visualization, motoneuron nuclei in the lumbosacral spinal cord, imaging of spinal cord, lumbosacral spinal cord, spinal cord, motoneuron nuclei, somatic motoneuron, immunolabelling, neuron, majority of neuron, autonomic preganglionic neuron, motor nuclei, imaging, cytoarchitectural context

Funders Acknowledgements:

NIH SPARC

Grant ID: 3OT2OD023872

Abstract

The whole-mount immunolabeling and clearing method (iDISCO) was used to visualize motoneuron nuclei in the lumbosacral spinal cord of adult rats. Imaging of spinal cord was performed on a light sheet microscope with a 12x lens. Choline acetyltransferase immunolabelling identified somatic motoneurons and autonomic preganglionic neurons, while NeuN immunolabelling identified a majority of neurons, to provide a cytoarchitectural context.

Materials

Materials

-  Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3641**
-  Dichloromethane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #320269**
-  Dibenzyl ether **Merck MilliporeSigma (Sigma-Aldrich) Catalog #108014**
-  Ethyl cinnamate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #112372**
-  1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #14190094**
-  Gelatin from porcine skin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G1890**
-  Hydrogen peroxide 30% **Merck Millipore (EMD Millipore) Catalog #822287.1000**
-  Saponin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S4521**
-  Thimerosal **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T5125**
-  Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML**
-  Anti-NeuN Antibody, clone A60 **Merck Millipore (EMD Millipore) Catalog #MAB377**
-  Goat anti-choline acetyltransferase antibody **Merck Millipore (EMD Millipore) Catalog #AB144P**
-  Cy3 donkey anti-mouse IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #715-165-150**
-  AF647 donkey anti-goat IgG **Jackson ImmunoResearch Laboratories, Inc. Catalog #705-605-147**

Equipment

Equipment	
Ultramicroscope II	NAME
Light sheet microscope	TYPE
Miltenyi Biotec	BRAND
NA	SKU
https://www.miltenyibiotec.com/AU-en/products/ultramicroscope-ii.html ^{LINK}	

Solutions

- PBS: phosphate-buffered saline, 0.1 M, pH 7.2
- DPBS: 1x Dulbecco's phosphate-buffered saline
- DPBS-T: 1x Dulbecco's phosphate-buffered saline containing 0.5% Triton X100

DPBSG-T: 1x Dulbecco's phosphate-buffered saline containing 0.2% gelatin, 0.5% Triton X-100 and 0.01% thimerosal

Primary antibodies

	A	B	C	D	E
	Abbreviation	Synonym	RRID	Host species	Dilution
	ChAT	Choline acetyltransferase	AB_11214092	Goat	1:500
	NeuN	Fox3	AB_2298772	Mouse	1:2000

Secondary antibodies

	A	B	C	D
	Tag-antibody	Host species	RRID	Dilution
	AF647 anti-goat	Donkey	AB_2340437	1:2000
	Cy3 anti-mouse	Donkey	AB_2340813	1:1000



Spinal cord preparation

30m

- 1 While immersed in phosphate buffered-saline (PBS), pH 7.2, trim nerve roots of fixed spinal cord to within approximately 2 mm of the spinal cord surface to facilitate the identification of segments later, following imaging.

Bleaching

1d

- 2 Wash samples in 1x Dulbecco's PBS (DPBS) (6 × 15 mins).
- 3 Dehydrate samples in a series of methanol in DPBS dilutions while on rotation at 12 rpm:
 1. 50% methanol in DPBS (1.5 h)
 2. 80% methanol in DPBS (1.5 h)
 3. 100% methanol (1.5 h)
- 4 Bleach samples overnight in 6% hydrogen peroxide in methanol at 4°C, protected from light.

Blocking

2d

- 5 Rehydrate samples in a series of methanol in DPBS dilutions while on rotation at 12 rpm:
 1. 100% methanol (2 × 1.5 h)
 2. 80% methanol in DPBS (1.5 h)
 3. 50% methanol in DPBS (1.5 h)
 4. DPBS (1.5 h)
- 6 Incubate samples in DPBS containing 0.2% gelatin, 0.5% Triton X-100 and 0.01% thimerosal (DPBSG-T) for 36 h while on rotation at 12 rpm

Primary antibody incubation

1w 3d

- 7 Incubate samples in primary antibody solution containing DPBSG-T with 0.1% saponin for 10 days at 37°C with agitation. Volume of solution need only be sufficient to cover the sample.

Secondary antibody incubation

5d

- 8 Wash spinal cords in 1x DPBS with 0.5% Triton X-100 (DPBST) (6 × 15 mins).



- 9 Incubate samples in secondary antibody solution containing DPBSG-T with 0.1% Saponin for 4 days at 37°C with agitation. Volume of solution need only be sufficient to cover the sample.

Dehydration and delipidation

1d 8h

- 10 Wash spinal cords in DPBST (6 × 15 mins).
- 11 Dehydrate spinal cords in a series of methanol in DPBS dilutions while on rotation at 12 rpm:
 1. 20% methanol in DPBS (1 h)
 2. 40% methanol in DPBS (1 h)
 3. 60% methanol in DPBS (1 h)
 4. 80% methanol in DPBS (1h)
 5. 100% methanol (2 × 1 h)
- 12 Incubate samples in a solution of 2/3 dichloromethane and 1/3 methanol overnight on rotation. Ensure that samples sink to the bottom of the vial at the end of this step, otherwise continue incubation in freshly made solution.
- 13 Incubate samples in 100% dichloromethane for 30 mins while on rotation at 12 rpm. Repeat this step until samples sink.

Clearing

2h

- 14 Incubate samples in dibenzyl ether until the samples have become clear. Ensure each vial is completely filled with dibenzyl ether to minimize sample oxidation as a result of large amounts of air in the vial. This process should not take longer than 2 h.

Storage

- 15 Store cleared samples in fresh dibenzyl ether. Keep away from light (wrap in foil and store in an opaque container).

Light sheet microscopy

- 16 Prior to visualization on a light sheet microscope, samples should be transferred into ethyl cinnamate, at least 3 h prior.
- 17 Remove sample from ethyl cinnamate and gently dry on a tissue. Affix sample to plastic mount using the minimum amount of super glue required. Tips:



- Avoid adhering the sample to the base via any region of the sample that is of interest; both the super glue and proximity to the plastic will reduce imaging quality in that area.
- Mount the sample perfectly in the middle; the light sheet microscope stage has limited mobility, particularly at higher magnifications.
- Orientate the sample such that the thinnest plane of the sample is perpendicular to the light sheet beams. The further light has to travel through a sample, the poorer the image quality.
- Orientate the sample so that the region of interest is facing as close to the lens as possible.

In this protocol, the spinal cord was mounted perpendicular to the light sheet beams, with the dorsal horn facing the lens, and the ventral side of L5 being the point of adherence to the mounting platform.

Protocol references

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., Tessier-Lavigne, M., 2014. iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. *Cell* 159, 896–910. <https://doi.org/10.1016/j.cell.2014.10.010>

Belle, M., Godefroy, D., Couly, G., Malone, S.A., Collier, F., Giacobini, P., Chédotal, A., 2017. Tridimensional Visualization and Analysis of Early Human Development. *Cell* 169, 161–173.e12. <https://doi.org/10.1016/j.cell.2017.03.008>