Oct 20, 2025 Version 2

Immunohistochemistry protocol for visualization of the corticospinal, corticobulbar,corticorubral, and rubrospinal tracts. V.2

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvw42yzlmk/v2

Jenny M. Michlich[1,2]

[1]Arizona State University; [2]Community College of Allegheny County

JMM

Jenny M. Michlich
Arizona State University, Community College of Allegheny Cou...

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvw42yzlmk/v2

Protocol Citation: Jenny M. Michlich 2025. Immunohistochemistry protocol for visualization of the corticospinal, corticobulbar,corticorubral, and rubrospinal tracts.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvw42yzlmk/v2Version created by **Jenny M. Michlich**

Protocol status: In development
We are still developing and optimizing this protocol

Created: October 18, 2025

Last Modified: October 20, 2025

Protocol Integer ID: 230171

Keywords: Immunohistochemistry, Motor systems, Corticospinal tract, Corticobulbar tract, Corticorubral tract, Rubrospinal tract, rubrospinal tract markers in neural tissue, rubrospinal tract marker, precise visualization of motor pathway component, rubrospinal tract, immunohistochemistry protocol for visualization, labeling glutamatergic terminal, identifying specific neuronal population, motor pathway component, vesicular glutamate transporter, dilution for developmental marker expression, glutamatergic terminal, immunohistochemistry protocol, specific neuronal population, neural tissue, developmental marker expression, conjugated secondary antibody, biotin complex

Funders Acknowledgements:
Graduate Prize Fellowship
Grant ID: Harvard University, Graduate School of Arts and Sciences

Abstract

This protocol describes the immunohistochemical (IHC) detection of corticospinal, corticobulbar, corticorubral, and rubrospinal tract markers in neural tissue. The procedure employs a three-marker approach to characterize motor system organization: PKC (protein kinase C) staining at 1:1500 dilution for identifying specific neuronal populations, VGLUT2 (vesicular glutamate transporter 2) at 1:200 dilution for labeling glutamatergic terminals, and OTX1 (orthodenticle homeobox 1) at 1:400 dilution for developmental marker expression. Tissue sections are processed through a standard avidin-biotin complex (ABC) method beginning with one-hour blocking in 5% normal serum, followed by overnight primary antibody incubation at 4°C in PBS with 5% blocking serum. After PBS washes, sections are incubated with biotin-conjugated secondary antibodies for 30-45 minutes, then treated with ABC reagent for 30 minutes. Visualization is achieved using diaminobenzidine (DAB) chromogenic detection under hood conditions. This systematic approach enables precise visualization of motor pathway components in these descending motor systems, facilitating anatomical and functional studies of descending motor control circuits.

Guidelines

Protocols for Solutions:

Peroxidase block (40 mL)

- 20 mL 0.2M phosphate buffer
- 8 mL methanol
- 80 µL Triton-X100
- 2 mL hydrogen peroxide
- Make up to 40 mL with ddH_2O

Blocking buffer

- 0.1M Phosphate buffer
- 0.3% Triton-X100
- 1% serum from the secondary antibody host species

Phosphate buffer (PBS, 1 L, pH 7.2):

- Prepare 800 mL of distilled water in a suitable container.
- Add 20.214 g of Sodium Phosphate Dibasic Heptahydrate to the solution
- Add 3.394 g of Sodium Phosphate Monobasic Monohydrate to the solution
- Adjust the solution to the final desired pH using HCl or NaOH
- Add distilled water until the volume is 1 L.

Phosphate-Buffered Saline (PBS, 1 L, pH 7.4):

- Prepare 800 mL of distilled water in a suitable container.
- Add 8 g of Sodium chloride to the solution
- Add 200 mg of Potassium Chloride to the solution
- Add 1.44 g of Sodium Phosphate Dibasic to the solution
- Add 245 mg of Potassium Phosphate Monobasic to the solution
- Adjust the solution to the desired pH.
- Add distilled water until the volume is 1 L.

Materials

Materials and reagents (PKCγ), corticospinal tract:

- Primary antibody, Anti-PKC Antibody (A-3): sc-17769
- Secondary antibody, m-IgG2a BP-HRP: sc-542731
- ImmunoCruz® ABC Kit: sc-516216
- Hydrogen peroxide: sc-203336
- 0.1 M Tris-HCl pH 7.6
- TBS or PBS
- Peroxidase block
- Blocking buffer
- 3,3'-Diaminobenzidine (DAB) kit

Materials and reagents (OTX1), corticobulbar tract:

- Primary antibody, OTX1 Antibody (3A5): sc-517000
- Secondary antibody, m-IgG2a BP-HRP: sc-542731
- ImmunoCruz® ABC Kit: sc-516216
- Hydrogen peroxide: sc-203336
- 0.1 M Tris-HCl pH 7.6
- TBS or PBS
- Peroxidase block
- Blocking buffer
- 3,3'-Diaminobenzidine (DAB) kit

Materials and reagents (VGLUT2), corticorubral and rubrospinal tract:

- Anti-VGLUT2 antibody, 8G9.2: ab79157
- Goat Anti-Mouse IgG H&L, HRP: ab205719
- ImmunoCruz® ABC Kit: sc-516216
- Hydrogen peroxide: sc-203336
- 0.1 M Tris-HCl pH 7.6
- 0.025% Triton X-100
- TBS or PBS
- Peroxidase block
- Blocking buffer
- 3,3'-Diaminobenzidine (DAB) kit

Safety warnings

3,3'-Diaminobenzidine (DAB) is a known cancer-causing agent. Please ensure proper handling methods when handling, including using a fume hood for all reactions with this compound.

Notes on dilutions

1 **Measurements and dilutions for PKCγ:**

- Dilutions: 1:1500 dilution in PBS and 5% normal blocking serum
- Primary: 0.0075 µL of stock solution in 2.5 mL
- Secondary: 0.0625 µL of stock solution in 2.5 mL
- Blocking serum: 0.125 mL of blocking reagent for 2.5 mL of solution

Measurements and dilutions for OTX1:

- Dilutions: 1:400 dilution in PBS with 5% normal blocking serum
- Primary: 0.015 µL of stock solution in 2.5 mL
- Secondary: 0.0625 µL of stock solution in 2.5 mL
- Blocking serum: 0.125 mL of blocking reagent for 2.5 mL of solution

Measurements and dilutions for VGLUT2:

- Dilutions: 1:200 dilution in PBS and 5% normal blocking serum
- Primary: 0.125 µL of stock solution in 2.5 mL
- Secondary: 0.25 µL of stock solution in 2.5 mL
- Blocking serum: 0.125 mL of blocking reagent for 2.5 mL of solution

Measurements and proportions (DAB):

- 0.3% H_2O_2
- 5 mL DI water
- 2 drops #1 solution from DAB kit
- 2 drops #3 solution from DAB kit
- 4 drops #2 solution from DAB kit

Methods

2 Perform antigen retrieval before commencing with immunostaining if necessary.

3 If using an HRP conjugate for detection, incubate the slides in 0.3% H_2O_2 in PBS for 15 min.

4 Wash with three changes of PBS for 5–7 minutes each.

5 Block in 10% normal serum with 1% BSA in PBS for 2 h at room temperature.

6 Drain slides for a few seconds (do not rinse) and wipe around the sections with tissue paper.

7 Apply the primary antibody diluted in PBS with 1% BSA.

8 Incubate overnight at 4°C with agitation, if possible.

9 Wash with three changes of PBS for 5–7 minutes each.

10 Incubate for 30–45 minutes with biotin-conjugated secondary antibody.

11 Wash with three changes of PBS for 5–7 minutes each.

12 Prepare the Avidin-Biotin Complex (ABC reagent) using the A and B reagents from the ABC Kit (sc-516216) by leaving the reagents out a room temperature for 30 minutes prior to use.

13 Mix 50 µl of each A and B reagent in a proportionate amount of PBS.

14 Incubate for 30 minutes with the ABC reagent.

15 Wash with three changes of PBS for 5–7 minutes each.

16 Incubate in hydrogen peroxide (0.3%) and DAB solution under a fume hood with a filtration system.

17 Wash with three changes of PBS for 5–7 minutes each

18 Store until mounting in PBS solution at 4°C.

Protocol references

1. Hadjivassiliou, G., Martinian, L., Squier, W. et al. The application of cortical layer markers in the evaluation of cortical dysplasias in epilepsy. Acta Neuropathol 120, 517–528 (2010). https://doi.org/10.1007/s00401-010-0686-x
2. Molnár, Z., & Cheung, A. F. P. (2006). Towards the classification of subpopulations of layer V pyramidal projection neurons. Neuroscience Research, 55(2), 105–115. https://doi.org/10.1016/j.neures.2006.02.008
3. Vigneault, É., Poirel, O., Riad, M., Prud'homme, J., Dumas, S., Turecki, G., Fasano, C., Mechawar, N., & El Mestikawy, S. (2015). Distribution of vesicular glutamate transporters in the human brain. Frontiers in Neuroanatomy, 9. https://doi.org/10.3389/fnana.2015.00023
4. Atoji, Y. (2011). Immunohistochemical localization of vesicular glutamate transporter 2 (vGluT2) in the central nervous system of the pigeon (Columba livia). Journal of Comparative Neurology, 519(14), 2887–2905. https://doi.org/10.1002/cne.22663
5. Saito, N., & Shirai, Y. (2002). Protein kinase Cγ (PKCγ): function of neuron-specific isotype. The journal of biochemistry, 132(5), 683-687.
6. Tanaka, C., & Saito, N. (1992). Localization of subspecies of protein kinase C in the mammalian central nervous system. Neurochemistry International, 21(4), 499–512. https://doi.org/10.1016/0197-0186(92)90081-2

Acknowledgements

The author would like to thank Dr. Erin Hecht for feedback on the development of this protocol.