Mar 10, 2025

# DIO-SPOTlight Immunohistochemistry v.250227

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7mo38gwz/v1

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Matt Oliver: Adapted from immunohistochemistry for p-eIF2a and eIF2a, v.230804;

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**DOI:** https://dx.doi.org/10.17504/protocols.io.q26g7mo38gwz/v1

**Protocol Citation:** Matt Oliver 2025. DIO-SPOTlight Immunohistochemistry v.250227. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7mo38gwz/v1

**Manuscript citation:**
Oliver, M. L., Caffall, Z. F., Eatman, C. B., Faw, T. D., & Calakos, N. (2024). DIO-SPOTlight Transgenic Mouse to Functionally Monitor Protein Synthesis Regulated by the Integrated Stress Response. *BioRxiv*, 2024.10.14.618312. https://doi.org/10.1101/2024.10.14.618312

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

**Created:** February 27, 2025

**Last Modified:** March 10, 2025

**Protocol Integer ID:** 123531

**Keywords:** ASAPCRN, DIO-SPOTlight IHC, spotlight immunohistochemistry, immunofluorescent labeling in pfa perfusion, using immunofluorescent labeling, spotlight image processing, immunofluorescent, pfa perfusion, spotlight, imaging

**Funders Acknowledgements:**
**ASAP**
Grant ID: ASAP-020607

## Abstract

Abstract
This protocol is to label several targets using immunofluorescent labeling in PFA perfusion-fixed, sagittal 40-µm thick slices, post-mortem mouse brain tissue.

Note: All steps using an orbital shaker were performed at low speed, gently shaking.

Partner protocols: *DIO-SPOTlight Confocal Imaging Acquistion v.250227* & *DIO-SPOTlight Image Processing and Analysis v.250227*

## Pre-staining tissue processing, washes, and blocking

1 Transfer 2-3 desired brain slices per well into a 24 well plate in PBS.

2 Quench remaining free aldehydes by exchanging PBS for **0.1M glycine in 1x PBS**. Incubate at room temperature (RT) for 30 minutes on an orbital shaker.

3 Perform alkaline antigen retrieval. Exchange quenching buffer for **1% NaOH in 1x PBS** for 20 minutes at RT on an orbital shaker.

3.1 Note: Make alkaline retrieval buffer fresh. Handle carefully.

4 Wash and permeabilize samples. Remove alkaline retrieval buffer and Wash 3x for 10 minutes at RT while on an orbital shaker in PBS-T (**1x PBS with 0.3% Triton X-100**).

5 Block samples in blocking buffer. Exchange wash buffer for blocking buffer (**10% normal donkey serum (NDS) + 1% BSA (w/v) in PBS-T**). Incubate samples for 1 hour at RT while on an orbital shaker.

## Antibody incubations

6 Prepare primary antibodies in dilution buffer (**5% normal donkey serum (NDS) + 1% BSA (w/v) in PBS-T**).

6.1 Anti-RFP (rabbit, 1:1000, Rockland Antibodies and Assays, cat no. 600-401-379)
Anti-GFP (chicken, 1:1000, Abcam, cat no. AB13970)
Anti-ChAT (goat, 1:200, Millipore, cat no. AB144P)

7 Exchange blocking buffer for dilution buffer containing primary antibodies. Incubate at 4C overnight while on an orbital shaker.

8 The following day, remove primary antibodies and wash samples 3x for 10 minutes each at RT in PBS-T wash buffer (**1x PBS with 0.3% Triton X-100**) while on an orbital shaker.

9 Prepare secondary antibodies in dilution buffer (**5% normal donkey serum (NDS) + 1% BSA (w/v) in PBS-T**).

9.1 Alexa Fluor 488 (Donkey-anti-Chicken) 1:500

Alexa Fluor 546 (Donkey-anti-Rabbit) 1:500
Alexa Fluor 647 (Donkey-anti-Goat) 1:500

10 Exchange wash buffer for dilution buffer containing secondary antibodies and incubate at RT while on an orbital shaker for 1 hour.

## Label nuclei, final washes, and slide mounting

11 Prepare Hoechst 33342 (10ug/ml) in PBS-T wash buffer **1x PBS with 0.3% Triton X-100**).

12 Exchange dilution buffer containing secondary antibodies for Hoechst 33342 in PBS-T and incubate for 10 minutes while on an orbital shaker at RT.

13 Wash 2 additional time in PBS-T 10 mins RT while on an orbital shaker.

14 Exchange PBS-T for PBS

15 Mount slices on microscope slides and seal with Vectashield Vibrance Mounting Media (cat no. H-1700).

16 Allow to dry overnight in the dark at RT and then store in fridge.