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🌐 Single-molecule Immunofluorescence Tissue Staining Protocol for Oligomer Imaging V.3

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details background fluorescence quenching and immunofluorescence staining of human brain tissue for oligomer imaging.

Attachments



[kb3ib25np.pdf](#)

154KB

Guidelines

- Use only clean bottles, flasks, magnetic stirrers, tweezers, weighing spatulas, measuring cylinders – everything should be cleaned, dried and covered if left on the side before next use.
- Everything should be handled with clean tweezers – gloves should not touch the samples, solutions and ideally anything placed into the solutions where the slides are.



Materials

Materials and Reagents

- Microtome
- Glass slides
- Xylene solution
- 100% alcohol
- Methanol
- Hydrogen peroxide (H₂O₂) solution
- Citrate buffer pH6
- Milli Q water
- Pressure cooker
- PBS
- Goat Serum 10%
- AlexaFluor antibody
- 0.1% Sudan black solution
- Vectashield
- Overslip



Immunofluorescences staining protocol for oligomer imaging

10m

- 1 Cut  8 μm tissue sections on a microtome and load onto glass slides.
- 2 Dry slides  Overnight at  37 °C – **cover over the top.** 10m 
- 3 Before staining commences keep slides for a few hours but ideally  Overnight at  60 °C . 10m 
- 4 De-wax sections through three pots of xylene solution. Use each fresh pots of xylene each time.
- 4.1 De-wax section through pot of xylene solution for  00:02:00 . (1/3) 2m
- 4.2 De-wax section through pot of xylene solution for  00:02:00 . (/23) 2m
- 4.3 De-wax section through pot of xylene solution for  00:02:00 . (3/3) 2m
- 5 Take sections through two pots of 100% alcohol.

Note

Use fresh pots each time – methylated spirits.
- 5.1 Take sections through pot of 100% alcohol for  00:02:00 . (1/2) 2m
- 5.2 Take sections through pot of 100% alcohol for  00:02:00 . (2/2) 2m



6 Put slides into methanol + hydrogen peroxide (H₂O₂) solution (100 ml: 1 ml) for

10m

00:10:00 at Room temperature **fresh pot each time.**

Note

This process will block any staining of endogenous peroxidase in the tissue sections.

7 Perform necessary antigen retrieval pretreatments by pressure cooking in citrate buffer.

7.1 Pressure cook sections in citrate buffer 6 for 00:10:00 at pressure (wait for it to release high pressure air) in **cleaned pressure cooker.**

10m

7.2 Cool under running **Milli Q** water – *never* under tap water.



8 Block non-specific antigen/antibody binding by placing sections in **PBS and Goat Serum 10%** for 00:30:00 . **NB Goat Serum is chosen for Goat-Raised antibodies.**

30m

9 Apply primary antibody for 01:00:00 at Room temperature .

1h

10 Wash in PBS with fresh buffer (**at least filtered if not cell culture grade**).



10.1 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (1/3)

5m

10.2 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (2/3)

5m

10.3 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (3/3)

5m

11 Apply secondary AlexaFluor antibody for 01:00:00 at Room temperature in the dark.

1h



12 Wash in PBS.



12.1 Wash  00:05:00 in PBS in the dark. (1/3)

5m

12.2 Wash  00:05:00 in PBS in the dark. (2/3)

5m

12.3 Wash  00:05:00 in PBS in the dark. (3/3)

5m

13 Add **filtered (0.22 μ m)** 0.1% Sudan black solution (0.1% sudan black/70% ethanol) for  00:10:00 at  Room temperature in the dark.

10m



14 Wash 2-3 times in 30% ethanol.



15 Mount section with Vectashield and coverslip (**Plasma cleaned slides**).

16 Take for imaging.

