Light microscopy immunoperoxidase staining protocol

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ABSTRACT

This protocol details the procedure of light microscopy immunoperoxidase staining protocol.

ATTACHMENTS

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Light microscopy immunoperoxidase staining protocol 1d 4h 30m

1 Select sections to process from the brain tissue bank.

2 

Wash sections thoroughly with a phosphate-buffered saline(PBS, [M]**0.01 Molarity (M)** , pH **7.4**) solution.

3 Treat the sections at **Room temperature** with a 1% sodium borohydride (NaBH4) solution in PBS for **00:20:00** . 20m

4 

Rinse sections thoroughly in PBS.

5 Pre-incubate sections for **01:00:00** at **Room temperature** in a solution containing 1% normal serum (from the species used to generate the secondary antibodies), 0.3% Triton X-100, and 1% bovine serum albumin (BSA) in PBS. 1h

6  1d

Incubate the sections for **24:00:00** at **Room temperature** in a solution containing the primary antibodies in 1% normal serum, 0.3% Triton X-100, and 1% BSA in PBS.

7 

Next day, thoroughly rinse the sections in PBS.

8  1h 30m

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Incubate the sections in a PBS solution containing the appropriate biotinylated secondary antibody (1:200; Vector Labs, Burlingame, CA, USA) combined with 1% normal serum, 0.3% Triton X-100, and 1% BSA for **01:30:00** at **Room temperature** .

9

Wash the sections in PBS.

10 1h 30m

Incubate the sections in an avidin-biotin-peroxidase complex (ABC; 1:100; Vector Labs, Burlingame, CA, USA) solution for **01:30:00** at **Room temperature** .

11

Rinse the sections in PBS twice followed by a third rinse in TRIS buffer (**0.05 Molarity (M)** ; **7.6**).

12 10m

Incubate the sections in a solution containing 0.025% 3,3′-diaminobenzidine tetrahydrochloride, **10 millimolar (mM)** imidazole, and 0.005% hydrogen peroxide in Tris buffer for **00:10:00** at **Room temperature** .

13

Rinse the sections with PBS.

14 Mount sections onto gelatin-coated slides, and coverslip with Permount.

15 Digitalize the slides with an Aperio ScanScope CS system (Aperio Technologies).

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