

FEB 16, 2024

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DOI:

dx.doi.org/10.17504/protocols.io. eq2lyjy4mlx9/v1

Protocol Citation: Aydin Alikaya, William Stauffer 2024. DAB Staining for GFP on Free-floating Fixed NHP Brain Tissue. protocols.io

https://dx.doi.org/10.17504/protoc ols.io.eq2lyjy4mlx9/v1

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

Created: Feb 07, 2024

(DAB Staining for GFP on Free-floating Fixed NHP Brain Tissue

Forked from Standard DAB Staining for Free-floating Fixed NHP Brain Tissue

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ABSTRACT

This protocol details the procedure for immunohistochemical 3,3'-Diaminobenzidine (DAB) staining of free-floating fixed brain tissue sections using the avidin/biotin ABC complex to detect Green Fluorescence Protein (GFP).

This protocol has been tested with free-floating non-human primate (NHP) brain tissue that has been fixed with 4% paraformaldehyde, cryoprotected with sucrose gradients, and cryo-sectioned → ← 50 µm

GUIDELINES

When using 6 well tissue culture plates [Falcon, 353046] to react individual sections, you will need **2+ mL** solutions for *each* well plate.

When using circular staining nets [e.g., Brain Research Laboratories #4115] to react multiple series of sections, you will need **50 mL** solutions for **each** staining net.

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Last Modified: Feb 16, 2024

PROTOCOL integer ID: 94871

Keywords: ASAPCRN,

Immunostaining, DAB, NHP Brain

Tissue

Funders Acknowledgement:

Aligning Science Across

Parkinson's

Grant ID: ASAP-020519

MATERIALS

Tissue:

NHP brain tissue sections (50 µm).

Materials/Equipment:

- Tissue culture plates or circular staining nets
- Orbital shaker
- Fume hood
- Nitrile Gloves
- Glass slides (charged or subbed)

Reagents:

- Phosphate-buffered saline (PBS)
- Hydrogen Peroxide: H₂O₂ (30%)
- Distilled water: dH₂O
- Primary Antibody: Thermo Fisher, Molecular Probes Cat# A11122, RRID: AB_221569
- Vectastain ABC-HRP Kit, Peroxidase (Rabbit IgG) (PK-4001, Vector Laboratories)
- Peroxidase (HRP) with Nickel (3,3'-diaminobenzidine) (SK-4100) (Vector Laboratories)

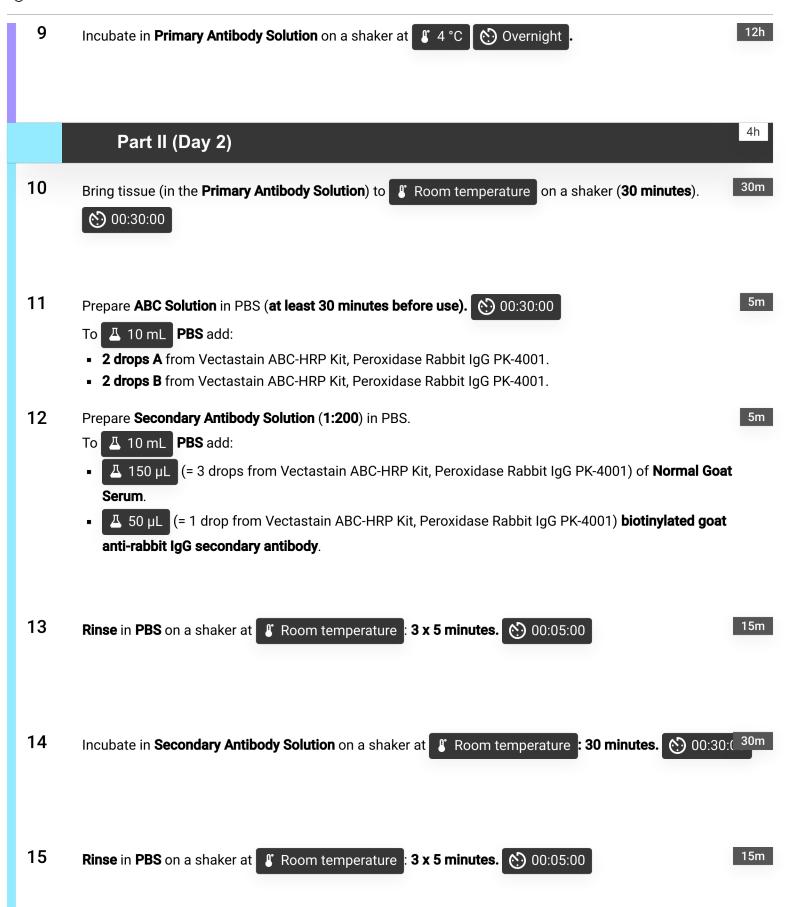
SAFETY WARNINGS

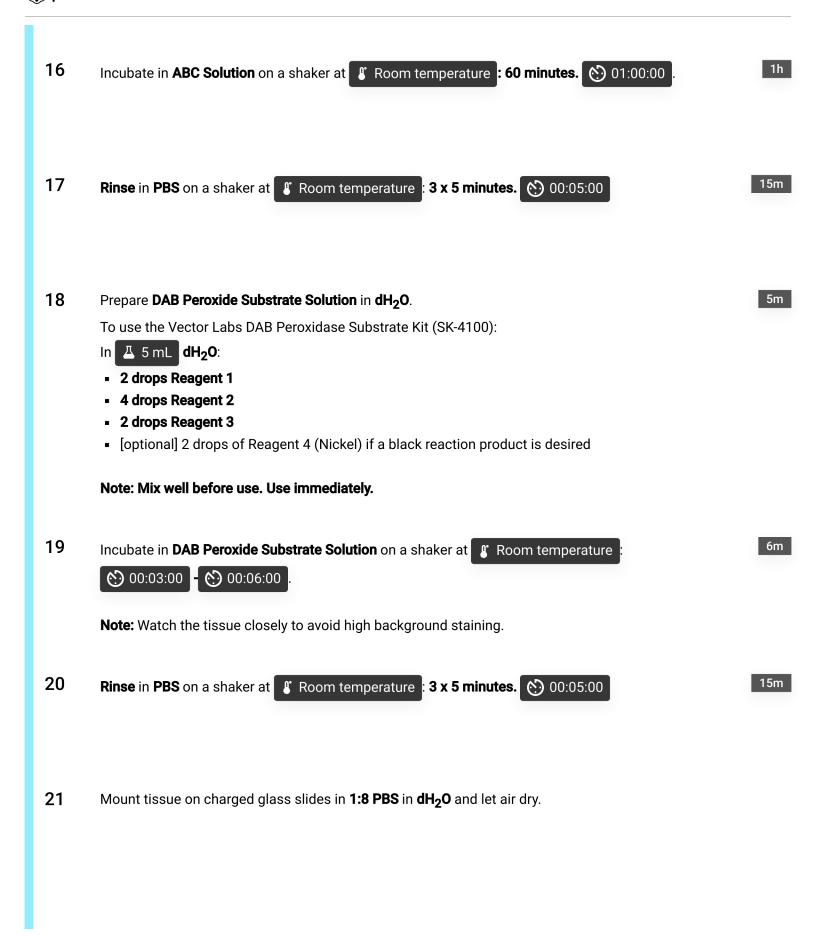
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Use appropriate care when using hydrogen peroxide (reactive, can cause skin/eye damage) and DAB (suspected carcinogen). Collect DAB solution for chemical waste disposal.

Part I (Day 1) Bring tissue to Room temperature in Phosphate Buffered Saline (PBS, pH 7.2-7.4) on an orbital shake 30m for 30 minutes. 00:30:00

2 Prepare Peroxide Solution (0.3 % H₂O₂) in dH₂O. 5m For \angle 10 mL **0.3%** H₂O₂ use: Д 100 μL **30% H₂O₂** Δ 9900 μL **dH₂O** 3 Prepare Normal Goat Serum Blocking Solution in PBS. 5m To Д 10 mL PBS add: Δ 150 μL Normal Goat Serum (= 3 drops of serum from Vectastain ABC-HRP Kit, Peroxidase Rabbit IgG PK-4001) 4 Prepare Primary Antibody Solution (rabbit anti-GFP) at 1:10000 dilution in PBS: 5m Δ 1 μL rabbit anti-GFP (Thermo Fisher, Molecular Probes Cat# A11122, RRID:AB_221569) **Δ** 9999 μL **PBS**. 5 15m Rinse in PBS on a shaker at | | Room temperature | 3 x 5 minutes. | (5) 00:05:00 Quench endogenous peroxide in **Peroxide Solution (0.3 H_2O_2)** on a shaker at \P Room temperature : 30 \P 30m 6 minutes. (5) 00:30:00 7 15m 8 Incubate in **Normal Goat Serum Blocking Solution** on a shaker at RT: **1 hour**. (5) 01:00:00 DO NOT RINSE after blocking serum.





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22 Rinse slides with **dH₂O** and let air dry (preferably in a hood).

23 Coverslip clean and dry slides with Cytoseal 60 (Thermo Fisher #830-16).