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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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We use this protocol and it's working

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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.

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: H_2O_2 (30%)
- Distilled water: dH_2O
- 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)

Troubleshooting

Safety warnings

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)

3h

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

2 Prepare **Peroxide Solution (0.3 % H_2O_2)** in **dH_2O**. — 5m

For 10 mL **0.3% H_2O_2** use:

- 100 µL **30% H_2O_2**
- 9900 µL **dH_2O**

3 Prepare **Normal Goat Serum Blocking Solution** in **PBS**. — 5m

To 10 mL **PBS** add:

- 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

- 1 µL **rabbit anti-GFP** (Thermo Fisher, Molecular Probes Cat# A11122, RRID:AB_221569)
- 9999 µL **PBS**.

5 **Rinse** in PBS on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 — 15m

6 Quench endogenous peroxide in **Peroxide Solution (0.3 H_2O_2)** on a shaker at Room temperature : **30 minutes.** 00:30:00 — 30m

7 **Rinse** in PBS on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 — 15m

8 Incubate in **Normal Goat Serum Blocking Solution** on a shaker at RT: **1 hour**. 01:00:00 — 1h

DO NOT RINSE after blocking serum.

9 Incubate in **Primary Antibody Solution** on a shaker at 4 °C Overnight . 12h

Part II (Day 2) 4h

10 Bring tissue (in the **Primary Antibody Solution**) to Room temperature on a shaker (**30 minutes**). 00:30:00 30m

11 Prepare **ABC Solution** in PBS (**at least 30 minutes before use).** 00:30:00 5m

To 10 mL **PBS** add:

- **2 drops A** from Vectastain ABC-HRP Kit, Peroxidase Rabbit IgG PK-4001.
- **2 drops B** from Vectastain ABC-HRP Kit, Peroxidase Rabbit IgG PK-4001.

12 Prepare **Secondary Antibody Solution** (**1:200**) in PBS. 5m

To 10 mL **PBS** add:

- 150 µL (= 3 drops from Vectastain ABC-HRP Kit, Peroxidase Rabbit IgG PK-4001) of **Normal Goat Serum**.
- 50 µL (= 1 drop from Vectastain ABC-HRP Kit, Peroxidase Rabbit IgG PK-4001) **biotinylated goat anti-rabbit IgG secondary antibody**.

13 **Rinse** in **PBS** on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 15m

14 Incubate in **Secondary Antibody Solution** on a shaker at Room temperature **: 30 minutes.** 00:30:00 30m

15 **Rinse** in **PBS** on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 15m

16 Incubate in **ABC Solution** on a shaker at Room temperature **: 60 minutes.** 01:00:00 . 1h

17 **Rinse** in **PBS** on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 15m

18 Prepare **DAB Peroxide Substrate Solution** in **dH_2O**. 5m

To use the Vector Labs DAB Peroxidase Substrate Kit (SK-4100):

In 5 mL **dH_2O**:

- **2 drops Reagent 1**
- **4 drops Reagent 2**
- **2 drops Reagent 3**
- [optional] 2 drops of Reagent 4 (Nickel) if a black reaction product is desired

Note: Mix well before use. Use immediately.

19 Incubate in **DAB Peroxide Substrate Solution** on a shaker at Room temperature : 6m

00:03:00 - 00:06:00 .

Note: Watch the tissue closely to avoid high background staining.

20 **Rinse** in **PBS** on a shaker at Room temperature : **3 × 5 minutes.** 00:05:00 15m

21 Mount tissue on charged glass slides in **1:8 PBS** in **dH_2O** and let air dry.

22 Rinse slides with **dH_2O** and let air dry (preferably in a hood).

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

Protocol references

https://vectorlabs.com/productattachments/protocol/VL_SK-4100_UserGuide_LBL02267.pdf
https://vectorlabs.com/products/vectastain-abc-hrp-kit-rabbit-igg