

# **VERSION 1**

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

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# Standard DAB Staining for Free-floating Fixed NHP Brain Tissue V.1

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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.

This protocol has been tested with free-floating non-human primate (NHP) and rodent (mouse, rat) brain tissue that has been fixed (10% formalin or 4% paraformaldehyde), cryoprotected (sucrose or glycerol gradients), and cryo-sectioned + 20 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 larger wells to react a series of sections, you will need **50 mL** solutions for **each** well.



PROTOCOL integer ID: 93354

Keywords: ASAPCRN,

Immunostaining, DAB, NHP Brain

Tissue

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**MATERIALS** 

#### Tissue:

Brain tissue sections (20 - 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<sub>2</sub>O<sub>2</sub> (3% or 30%)
- Distilled water: dH<sub>2</sub>O
- Primary Antibody
- Secondary Antibody (to match the host of the primary antibody)
- Normal Serum Blocking Solution (e.g., Normal Horse Serum, S-2000-20; Normal Goat Serum, S-1000-20)
- Vectastain Elite ABC Peroxidase Kit (Standard) (PK-6100) (Vector Laboratories)
- ABC-HRP Kit

#### Examples:

Vectastain ABC-HRP Kit, Peroxidase (Mouse IgG) (PK-4002, Vector Laboratories) Vectastain ABC-HRP Kit, Peroxidase (Rabbit IgG) (PK-4001, Vector Laboratories)

DAB Substrate Kit

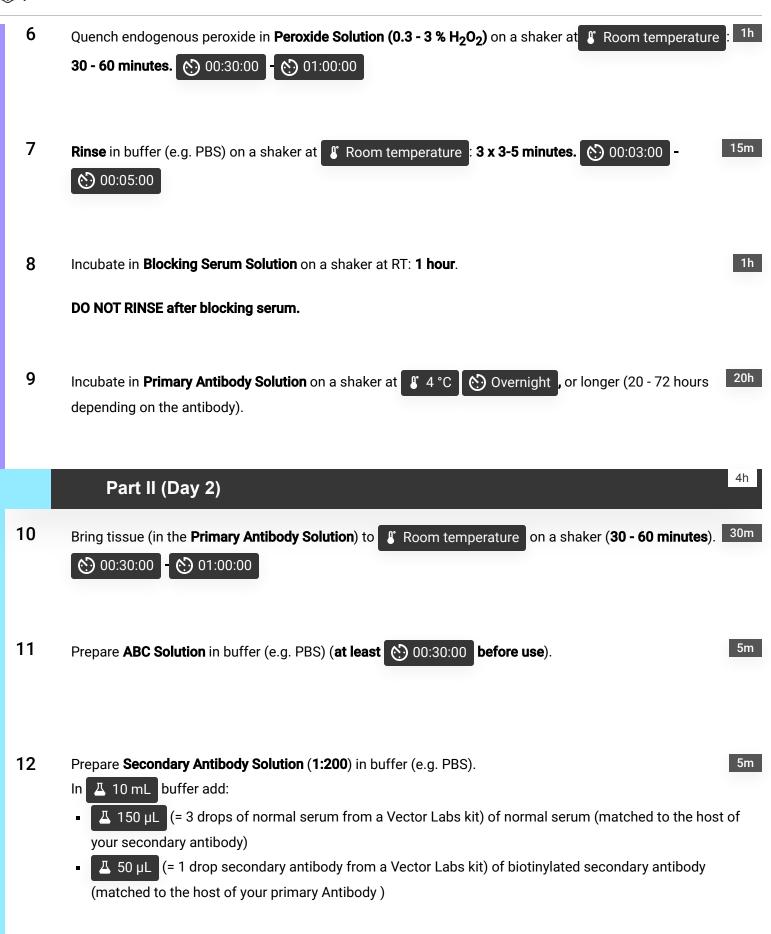
### Examples:

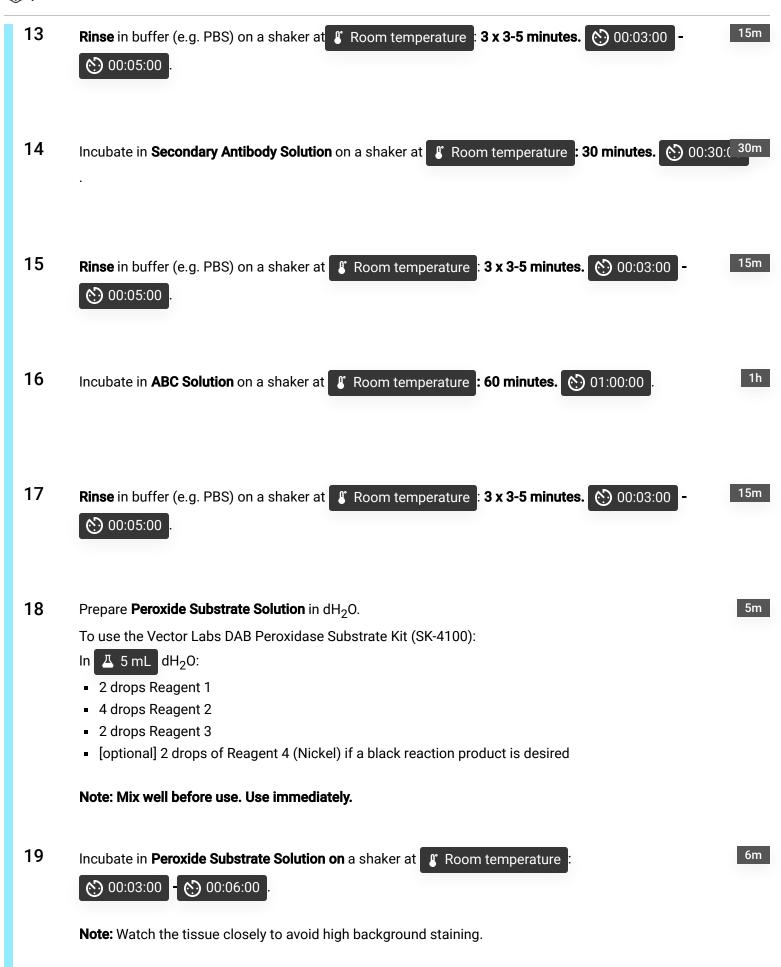
Peroxidase (HRP) with Nickel (3,3'-diaminobenzidine) (SK-4100) (Vector Laboratories) ImmPACT DAB (SK-4105)

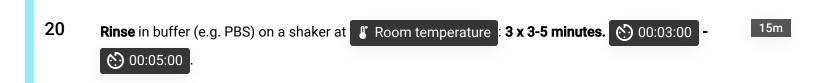
#### SAFETY WARNINGS

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) Bring tissue to § Room temperature in buffer (e.g., Phosphate buffered saline, PBS) on an orbital shake 30m 1 00:30:00 2 Prepare **Peroxide Solution (0.3 - 3 % H<sub>2</sub>O<sub>2</sub>)** in dH<sub>2</sub>O. 5m E.g., for $\angle$ 10 mL 0.3% H<sub>2</sub>O<sub>2</sub> use: Д 100 µL 30% H<sub>2</sub>O<sub>2</sub> Д 9900 μL dH<sub>2</sub>O 3 Prepare **Blocking Serum Solution** (e.g. Normal Horse, Normal Goat Serum) using a serum that matches the 5m host of the secondary antibody (e.g. Normal Horse Serum for a Horse anti-Mouse secondary, Normal Goat Serum for a Goat anti-Rabbit secondary). E.g., in Д 10 mL buffer (PBS) add: <u>μ 150 μL</u> normal serum (or 3 drops of normal serum if using an ABC kit, e.g. Vectastain ABC-HRP Kit, Peroxidase Mouse IgG PK-4002, Rabbit IgG PK-4001) 4 Prepare **Primary Antibody Solution** at the appropriate dilution in buffer (e.g., 1:1000 in PBS). 5m 5 15m Rinse in buffer (e.g. PBS) on a shaker at Room temperature: 3 x 3-5 minutes. (5) 00:03:00 00:05:00







- Mount tissue on glass slides (subbed or charged) in 1:8 buffer in dH<sub>2</sub>O and let air dry.
- Rinse slides with dH<sub>2</sub>O and let air dry (preferably in a hood).
- 23 Coverslips clean and dry slides with Cytoseal 60 (Thermo Fisher #830-16).