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## TH DAB staining

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1 Works for me



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### ABSTRACT

This protocol details the staining procedure for TH DAB staining.

### ATTACHMENTS

[ds8ybjt7.pdf](#)

### DOI

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### KEYWORDS

TH DAB staining, TBST, ASAPCRN

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## OWNERSHIP HISTORY

Jun 16, 2021  Urmilas

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50825

## MATERIALS TEXT

### Materials:

 Tyrosine hydroxylase polyclonal antibody **Enzo Life**

**Sciences Catalog #BML-SA497-0100**

 Goat Anti-Rabbit IgG Biotin Conjugate **Millipore**

**Sigma Catalog #OS03B**

 VECTASTAIN Elite ABC HRP Kit (Peroxidase, Standard) **Vector**

**Laboratories Catalog #PK-6100**

## TH DAB staining

6h 18m

1




Wash a full set of sections (6-8 sections) in a well in PBS, 5 min × 3.

1.1




5m

Wash a full set of sections (6-8 sections) in a well in PBS,  **00:05:00** (wash 1/3)  
(if stored in cryoprotective solution).

1.2




5m

Wash a full set of sections (6-8 sections) in a well in PBS,  **00:05:00** (wash 2/3)  
(if stored in cryoprotective solution).

1.3







5m

Wash a full set of sections (6-8 sections) in a well in PBS,  **00:05:00** (wash 3/3)  
(if stored in cryoprotective solution).

30m



2 

Incubate sections in 1% H<sub>2</sub>O<sub>2</sub> (  1 mL 30% H<sub>2</sub>O<sub>2</sub> in  29 mL TBST),  00:30:00 at  Room temperature .

3 

Wash sections in TBST 5 min × 3.

3.1 

5m

Wash sections in TBST  00:05:00 (wash 1/3).

3.2 

5m

Wash sections in TBST  00:05:00 (wash 2/3).

3.3 

5m

Wash sections in TBST  00:05:00 (wash 3/3).

4 Block sections in 5% NGS/TBST,  01:00:00 at  Room temperature .

1h

5  

Incubate sections in rabbit anti-TH (#SA-497), 1:3000 in TBST,  4 °C  Overnight .


6 

Wash sections in TBST, 10 min × 3.

6.1 

10m

Wash sections in TBST,  00:10:00 (wash 1/3).

6.2 

10m

Wash sections in TBST, ⌚00:10:00 (wash 2/3).

6.3 

10m

Wash sections in TBST, ⌚00:10:00 (wash 3/3).

7 


1h

Incubate sections in anti-rabbit biotinylated (#OS03B), 1:3000, ⌚01:00:00 at  
📍 **Room temperature** .

8 Prepare ABC (Avidin-Biotin-Complex, Vectastain ABC Kit, # PK-6100): 1 drop A and 1 drop B in  
📄 **2.5 mL** TBST, at least 30 min before use.

9 

Wash sections in TBST, 10 min × 3.

9.1 

10m

Wash sections in TBST, ⌚00:10:00 (wash 1/3).

9.2 

10m

Wash sections in TBST, ⌚00:10:00 (wash 2/3).

9.3 


10m

Wash sections in TBST, ⌚00:10:00 (wash 3/3).

10 

30m

Incubate sections in ABC, ⌚00:30:00 at 📍 **Room temperature** .

11 Prepare DAB solution (Sigma FAST, DAB Peroxidase Substrate Tablet Set), 1 DAB and 1 Urea Hydrogen Peroxide tablet in  15 mL distilled water.

12 

Wash sections in TBST, 10 min × 3.

12.1 

10m

Wash sections in TBST,  00:10:00 (wash 1/3).

12.2 

10m

Wash sections in TBST,  00:10:00 (wash 2/3).

12.3 

10m

Wash sections in TBST,  00:10:00 (wash 3/3).

13 

3m

Incubate sections in  1 mL DAB,  00:01:00 -  00:02:00 .

14 

Wash sections in TBST, 5 min × 3.

14.1 

5m

Wash sections in TBST,  00:05:00 (wash 1/3).

14.2 

5m

Wash sections in TBST,  00:05:00 (wash 2/3).

14.3 

5m

Wash sections in TBST, 🕒 **00:05:00** (wash 3/3).

15 Mount the sections on positive charged plus slides.