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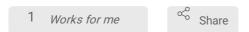
## TH DAB staining

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**ABSTRACT** 

This protocol details the stating procedure for TH DAB staining.

**ATTACHMENTS** 

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**KEYWORDS** 

TH DAB staining, TBST, ASAPCRN

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## **OWNERSHIP HISTORY**

Jun 16, 2021 Urmilas

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PROTOCOL INTEGER ID

50825

MATERIALS TEXT

Materials:

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

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2

2 ===

Incubate sections in 1%  $H_2O_2$  (  $\blacksquare 1$  mL 30%  $H_2O_2$  in  $\blacksquare 29$  mL TBST),  $\odot 00:30:00$  at 8 Room temperature .

3

Wash sections in TBST 5 min × 3.

3.1

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

3.2

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

3.3

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

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

1h

5m

5m

5m

5

6

Wash sections in TBST, 10 min × 3.

6.1 6.1

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

6.2 A

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

6.3 A

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

Incubate sections in anti-rabbit biotinylated (#OS03B), 1:3000,  $\bigcirc$  **01:00:00** at **8 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 Po

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

9.2 Po

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

9.3 Po

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

30m

Incubate sections in ABC, © 00:30:00 at 8 Room temperature.

10

Prepare DAB solution (Sigma FAST, DAB Peroxidase Substrate Tablet Set), 1 DAB and 1 Urea 11 Hydogen Peroxide tablet in **15 mL** distilled water. 12 Wash sections in TBST, 10 min × 3. 10m 12.1 Wash sections in TBST, © 00:10:00 (wash 1/3). 10m 12.2 Wash sections in TBST, © 00:10:00 (wash 2/3). 10m 12.3 Wash sections in TBST, © 00:10:00 (wash 3/3). 3m 13 Incubate sections in **1 mL** DAB, **00:01:00 - 00:02:00**. 14 Wash sections in TBST, 5 min × 3. 5m 14.1 Wash sections in TBST, © 00:05:00 (wash 1/3). 5m 14.2 Wash sections in TBST, © 00:05:00 (wash 2/3). 5m 14.3

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

15 Mount the sections on positive charged plus slides.