

Feb 15, 2022

Peroxidase/DAB protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.b44fqytn>

Christiana.bjorkli¹

¹The Norwegian University of Science and Technology

Sandvig lab



Christiana.bjorkli

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.b44fqytn>

Protocol Citation: Christiana.bjorkli 2022. Peroxidase/DAB protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.b44fqytn>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: February 15, 2022

Last Modified: February 15, 2022

Protocol Integer ID: 58215

Keywords: dab protocol peroxidase, dab protocol for chromogen labelling, peroxidase, chromogen labelling, dab protocol

Abstract

Peroxidase/DAB protocol for chromogen labelling



- 1 Leave sections in [IM] 0.125 Mass Percent **phosphate buffer (PB)** at 60 °C 2h
- 1.1 Dilute 0,4M **PB**. Store in refrigerator for up to 1 week.
100 ml: 31,25 ml [IM] 0.4 Mass Percent **PB** + 68,75 ml **H₂O**
- 2 Rinse sections 2 × 10min in **PB** 20m
- 3 Rinse sections 1 × 10 min in 0,5% **TBS-Tx** 10m
- 3.1 **Tris**: 3,03 gram/500 mlH₂O
NaCl: 4,48 gram/500 mlH₂O
Triton-X-100: 2,5 ml/500 mlH₂O
Use HCl to adjust the 8 . Store in refrigerator for up to one week.
- 4 Incubate with 10% goat serum in **TBS-Tx** 30m
- 5 **Incubate with primary antibody** (AT8; MAP2) 1:1000 in **TBS-Tx** with 5% goat serum, overnight in refrigerator 4 °C 12h
- 6 Rinse 3 × 10 min in **TBS-Tx** 30m

Note

Mix secondary antibody during first wash, and start dissolving DAB during second wash. Leave DAB on heated stirrer for 2 hours, then leave on bench.
- 6.1 **DAB**
Dissolve 1 tablet (10 mg) in 15 ml **Tris-HCl** by leaving it on a stirrer with heat (max 50 °C) for about 2 hours. Add 12 µL **H₂O₂** just before use and filtrate.

For dissolving the **DAB** use a brain cup (or any other cup you can throw away)



For filtrating the **DAB** use a disposable syringe with filter.

Safety information

Remember that **DAB** and **H₂O₂** are hazardous chemicals. Wear gloves, work in the hood, and put the **DAB**-solution and the first **HCl**-waste in the **DAB** waste-bottle.

- 7 **Incubate with secondary antibody**, biotinylated goat anti-mouse, 1:500 in **TBS-Tx** with 5% goat serum, in room temperature

1h 30m

Note

Mix ABC right before next step

- 7.1 From the **ABC**-kit, put 1 drop of **solution A** and 1 drop of **solution B** in 5 mL **TBS-Tx**. Mix well and leave on the bench for 30 min before use.

- 8 Rinse sections 3 × 10 min in **TBS-Tx**

30m

- 9 **Incubate with ABC** in room temperature

1h 30m

- 10 Rinse 3 × 10 min in **TBS-Tx**

30m

- 11 Rinse 2 × 5 min in **Tris-HCl**

10m

- 12 **Incubate with DAB**

10m

- 13 Rinse 2 × 5 min in **Tris-HCl**

10m

- 14 Mount sections in **Tris-HCl** on Superfrost slides and let them dry



15 Coverslip with **Xylene** and **Entellan**