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# Peroxidase/DAB protocol

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1

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Peroxidase/DAB protocol for chromogen labelling

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1 Leave sections in **[M]0.125 Molarity (M) 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 **[M]0.4 Molarity (M) PB** + 68,75 ml **H<sub>2</sub>O**

2 Rinse sections 2 x 10min in **PB**

20m

3 Rinse sections 1 x 10 min in 0,5% **TBS-Tx**

10m

3.1 **Tris**: 3,03 gram/500 mlH<sub>2</sub>O  
**NaCl**: 4,48 gram/500 mlH<sub>2</sub>O  
**Triton-X-100**: 2,5 ml/500 mlH<sub>2</sub>O

Use HCl to adjust the **pH** 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 x 10 min in **TBS-Tx**

30m

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 **H<sub>2</sub>O<sub>2</sub>** 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.



Remember that **DAB** and **H<sub>2</sub>O<sub>2</sub>** 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 <sup>1h 30m</sup>

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 x 10 min in **TBS-Tx** <sup>30m</sup>
- 9 **Incubate with ABC** in room temperature <sup>1h 30m</sup>
- 10 Rinse 3 x 10 min in **TBS-Tx** <sup>30m</sup>
- 11 Rinse 2 x 5 min in **Tris-HCl** <sup>10m</sup>
- 12 **Incubate with DAB** <sup>10m</sup>
- 13 Rinse 2 x 5 min in **Tris-HCl** <sup>10m</sup>
- 14 Mount sections in **Tris-HCl** on Superfrost slides and let them dry
- 15 Coverslip with **Xylene** and **Entellan**

