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 We use this protocol and it's working

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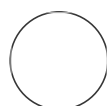
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# Neuromelanin staining (Fontana-Masson staining)+ TH-DAB staining on midbrain organoids

In 1 collection

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## ABSTRACT

Fontana-Masson staining is a silver staining technique that is commonly used to identify melanin-containing cells. We combined it with DAB-TH staining that works by using an antibody to detect the presence of TH, followed by a reaction with a substrate (DAB) that results in the formation of a brown-colored product at the site of the antigen-antibody interaction.

## ATTACHMENTS

[676-1423.docx](#)

## MATERIALS

### Materials

- methanol (MeOH)
- 3% hydrogen peroxide
- PBS
- Triton-X 0.2%
- DAB solution
- VECTASTAIN® Elite® ABC-HRP Kit

Fontana-Masson Stain Kit **Merck MilliporeSigma (Sigma-Aldrich) Catalog**  
**#HT200-1KT**

# Neuromelanin staining

5h 35m 30s

1



Incubate human midbrain organoid sections in a fresh solution of 3:1 methanol (MeOH)/3% hydrogen peroxide at Room temperature for 00:20:00 .

20m

2



Wash the slides and block it.

2.1

Wash the slides in PBS for 00:05:00 . (1/3)

5m

2.2

Wash the slides in PBS for 00:05:00 . (2/3)

5m

2.3

Wash the slides in PBS for 00:05:00 . (3/3)

5m

2.4

Then, block with NGS 10% in PBS+ Triton-X 0.2% for 01:00:00 at Room temperature .

1h

3



Apply primary antibodies in NGS 5% in PBS+ Triton-X 0.2% solution Overnight at 4 °C .

1h


4



Next, wash slides.

4.1 Wash the slides in PBS for  00:05:00 . (1/3)



5m

4.2 Wash the slides in PBS for  00:05:00 . (2/3)



5m

4.3 Wash the slides in PBS for  00:05:00 . (3/3)

5m

4.4 Apply secondary antibodies to NGS 5% in PBS+ Triton-X 0.2% solution for  01:00:00 at  Room temperature .

1h

5 Prepare ABC solution from Vectastain according to the manufacturer's instructions (VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase (Standard) PK-6100) and apply to sections for  01:00:00 at  Room temperature .

1h

6 Wash the slides.




6.1 Wash the slides in PBS for  00:05:00 . (1/3)



5m



6.2 Wash the slides in PBS for  00:05:00 . (2/3)

5m

6.3 Wash the slides in PBS for  00:05:00 . (3/3)

5m

7 Prepare DAB solution according to the manufacturer's instructions by diluting in  50 mL of 1x PBS with  50  $\mu$ L of 3% H<sub>2</sub>O<sub>2</sub>.

8 Apply DAB solution to the sections at  Room temperature for 30 seconds to  00:12:00 depending on when the visible reaction occurred.

12m

9 For the visualization of neuromelanin, use the Fontana-Masson stain kit, according to the manufacturer's instructions. (Fontana-Masson Stain Kit; Sigma–Aldrich-HT200).

10 Eventually mount slides with synthetic resin.