

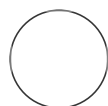


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🌐 Neuromelanin staining (Fontana-Masson staining)-DAB staining on midbrain organoids

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We use this protocol and it's working

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ABSTRACT

Fontana-Masson staining is a silver staining technique that is commonly used to identify melanin-containing cells. That was combined 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

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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

Keywords: Fontana-Masson
Stain Kit, Neuromelanin
staining

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 μ L of 3% H₂O₂.

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.