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We use this protocol and it's working

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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

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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**

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 µL of 3% H_2O_2.

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.