



Mar 27, 2023

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

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygx98mdg8j/v1>

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Protocol Citation: michela.deleidi , Maria Jose Perez J., Hariam Raji, Pascale Baden, Federico Bertoli 2023. Neuromelanin staining (Fontana-Masson staining)-DAB staining on midbrain organoids. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.kxygx98mdg8j/v1>

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Protocol status: Working

We use this protocol and it's working

Created: March 17, 2023

Last Modified: March 27, 2023

Protocol Integer ID: 78956

Keywords: Fontana-Masson Stain Kit, Neuromelanin staining , staining technique, melanin, th staining, dab, antibody, antigen, containing cell, cell, colored product

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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13KB

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% 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.