**Neuromelanin staining (Fontana-Masson staining)-DAB staining**

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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.

**Protocol**

Human midbrain organoid sections were incubated in a fresh solution of 3:1 methanol (MeOH)/3% hydrogen peroxide at room temperature for 20 minutes. Slides were then washed in PBS 1X 3 times for 5 minutes and blocked with NGS 10% in PBS+ Triton-X 0.2% for 1 h at room temperature. Primary antibodies were applied in NGS 5% in PBS+ Triton-X 0.2% solution overnight at 4°C. Next, slides were washed in 1X PBS 3 times for 5 minutes, and secondary antibodies were applied to NGS 5% in PBS+ Triton-X 0.2% solution for 1 h at room temperature. ABC solution from Vectastain was prepared according to the manufacturer’s instructions (VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase (Standard) PK-6100) and applied to sections for 1 h at room temperature. Slides were then washed in 1X PBS 3 times for 5 minutes. DAB solution was prepared according to the manufacturer’s instructions by diluting in 50 ml of 1x PBS with 50 L of 3% H2O2. DAB solution was applied to the sections at room temperature for 30 seconds to 12 minutes depending on when the visible reaction occurred. For the visualization of neuromelanin, the Fontana-Masson stain kit (Fontana-Masson Stain Kit; Sigma‒Aldrich-HT200) was used. After DAB staining, slides were incubated in warmed ammonium silver solution at 58–60°C for 30 minutes, according to the manufacturer’s instructions. Slices were toned in Gold Chloride Solution for 30 seconds and then placed in Sodium Thiosulfate Solution for 1–2 minutes. Slides were eventually mounted with synthetic resin.