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Neuromelanin Processing and Image Acquisition

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ABSTRACT

Protocol for preparing post-mortem tissue for intracellular NM quantification.

Keywords: ASAPCRN

Tissue Processing

1 Formalin-fixed paraffin-embedded tissue blocks from the pontine and midbrain regions were sectioned at 6µm, collected onto adhesive microscope slides and allowed to dry in the oven at 37°C for 48 hours.

2 Slides were incubated in the oven at 60°C for 1 hour to melt the paraffin.

3 To remove the paraffin, sections were submerged in HistoChoice Clearing Agent (Sigma-Aldrich, H2779) for 2 x 7 minutes, followed by rehydration in decreasing ethanol concentrations (100% ethanol for 2 x 3 minutes, 95% ethanol for 3 minutes, 70% ethanol for 3 minutes) and distilled H2O for 3 minutes.

4 Slides were submerged in 1xPBS and coverslipped using Fluorescence Mounting Medium (DAKO, S302380-2).

Image Acquisition

5 Sections were scanned using 20x objective (NA=0.8) with pre-set focusing and exposure parameters for optimal NM signal quality with an automated Slide Scanner (SLIDEVIEW VS200, Tokyo, Japan).

6 Identical parameters were applied to each scanned section to ensure consistency in capturing NM.

7 Once the scan was completed, sections were submerged in 1xPBS to remove coverslips and continue with IF processing.