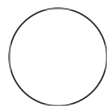




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## Determination of NM Concentration

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### ABSTRACT

This is the protocol for determining neuromelanin concentration and data.

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**Protocol status:** Working

We use this protocol and it's working

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**Keywords:** ASAPCRN

- 1 Place SNpc tissue in a plastic tube and carefully ground it. Weigh 10 mg of tissue for each sample, and place it in a 5 mL glass tube.

- 2 In each tube, add 1.5 mL of pH 7.4 phosphate buffer (50mM), shake, and centrifuge at 9000 x g for 30 mins. Discard the supernatant.
- 3 Wash with phosphate buffer and repeat once more.
- 4 Add 1.5ml of Tris buffer (50 mM, pH 7.4) solution, containing sodium dodecyl sulfate (5 mg/ml) and 0.2 mg/ml proteinase K to the pellet of each sample. Incubate the pellet by shaking in this solution for 2 hours at 37°C.
- 5 Centrifuge the suspension of pigment at 9000 x g for 30 minutes.
- 6 Wash the pellet with 1.5 ml of NaCl solution (9 mg/ml) and 1.5 ml of water. Centrifuge at 9000 x g for 30 minutes.
- 7 Dissolve the NM residue in 1 ml of 1M NaOH at 80°C for 1 hour.
- 8 Centrifuge this solution and transfer the supernatant into a quartz cuvette, measure the absorbance at 350 nm.
- 9 To run calibration curves dissolve known amounts of NM (ranging from 1 – 30 µg) in 1 ml of 1 M NaOH at 80°C for 1 hour.

- 10** NM value was the average from 2-3 replicates. The final values of NM concentrations are expressed as  $\mu\text{g}/\text{mg}$  dry tissue.