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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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 (50 mM), shake, and centrifuge at 9000 x g for 30 mins. Discard the supernatant.

3. Wash with phosphate buffer and repeat once more.

4. Add 1.5 mL 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 37ºC.

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 80ºC for 1 hour.

8. Centrifuge this solution and transfer the supernatant into a quartz cuvette, measure the absorbance at 350 nm.
To run calibration curves dissolve known amounts of NM (ranging from 1 – 30 µg) in 1 ml of 1 M NaOH at 80ºC for 1 hour.

NM value was the average from 2-3 replicates. The final values of NM concentrations are expressed as µg/mg dry tissue.