Determination of NM Concentration

Xiqun Chen[1,2]

[1]Massachusetts General Hospital;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Daniel Choo

APR 06, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj8wxxgk5/v1

Protocol Citation: Xiqun Chen 2023. Determination of NM Concentration. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj8wxxgk5/v1

Protocol status: Working
We use this protocol and it's working

Created: Apr 06, 2023

Last Modified: Apr 06, 2023

PROTOCOL integer ID: 80139

Keywords: ASAPCRN

ABSTRACT

This is the protocol for determining neuromelanin concentration and data.

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

9 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.

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