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# Isolation of pure neuromelanin extracts from cultured TR5TY6 neuroblastoma cells

In 1 collection

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**We use this protocol and it's working**

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## Abstract

Isolation of pure neuromelanin extracts from cultured TR5TY6 neuroblastoma cells

## Cell culture

1 Seed TR5TY6 cells in 12 cm diameter plates using 20 ml of medium per plate. 10 big plates will yield approximately 1-2 mg of pigment, depending on confluence.

2 Next day, induce tyrosinase expression by changing the medium for a new one containing 2 ug/ml doxycycline (stock 2 mg/ml).

3 Incubate the cells for 6 days. DO NOT CHANGE MEDIUM during this time, since neuromelanin accumulates not only in living cells, but also in dying cells and media.

4 Harvest the cells by using a cell scraper and collect them together with the medium in 50 ml tubes. Wash all the plates with extra medium (10 ml) and add it to the tubes.

5 Centrifuge at 1800 rpm for 7 min and discard supernatant.

6 Wash pellets with 10 ml PBS

7 Centrifuge again at 1800 rpm for 7 min, discard the supernatant and freeze immediately at -80ºC.

## Neuromelanin isolation

8 Thaw cell pellet at RT

9 Wash in 10 ml of **0.05 N phosphate buffer pH 7.2** (2-3 pellets)

10 Centrifuge at 10,000 x g for 15 min at RT

11 Repeat washing and centrifugation steps

12 Add 10 ml of **5 mg/ml SDS** (freshly added during the previous centrifuge) in 75 mM Tris, pH 7.5

13 Sonicate (until total resuspension) and incubate for 3 h at 37°C in a shaking water bath.

14 Centrifuge at 10,000 x g at RT for 30 min and remove the supernatant.

15 Add 2 ml (2-3 pellets) **of 5 mg/ml SDS** in 75 mM Tris, pH 7.5 with **0.33 mg/ml proteinase K** (82.5ul in 5ml) and incubate for 3 h at 37°C

16 Transfer the samples to Eppendorf tubes, centrifuge at 10,000 x g at RT for 30 min and remove the supernatant.

17 Wash pellets with 1 ml **0.9% NaCl**

18 Centrifuge at 10,000 x g for 30 min at RT and discard the supernatant.

19 Resuspend each pellet in **1 ml of MQ $H_2O$** (use the same ml for every pellet, taking it form one tube to the next one), pool them together in one tube and separate 25 ul of the resuspended sample for nanodrop quantification. Store it at 4ºC.

20 Centrifuge at 10,000 x g at RT for 30 min and remove the supernatant.

21 Rewash pellets in 1 ml of **methanol. DO NOT resuspend the pellet.**

22 Centrifuge at 10,000 x g at RT for 30 min and discard the supernatant.

23 Wash with 1 ml of **hexane DO NOT resuspend the pellet.**

24 Centrifuge at 10,000 x g at RT for 30 minutes.

25 Dry isolated NM under fume hood (be careful not to overdry the sample), fast-freeze by liquid nitrogen immersion and store at -80°C.