

## Melanin quantitation (Ando-sensei@OUS,

<https://www.sciencedirect.com/science/article/pii/S0022202X1535689X?via%3DiHub>

PMID: 22189785)

### 6 well culture

↓ melanin addition

Collect cells (~48h)

↓ Resusp in 100 $\mu$ l of DW

↓ +Add 400 $\mu$ l of EtOH:(Et)<sub>2</sub>O=1:1 sol'n.

Leave for 5min, @r.t.

↓ Centrifuge at 1000g, 5min

Sup (PNS)

↓ Centrifuge at 15000g, 5min

Collect ppt (remove sup)

↓ +Add 110 $\mu$ l of 10%DMSO in 1M NaOH

Incubate at 80°C, 10min, mixing

Centrifuge

Take sup 100 $\mu$ l

↓ Measure Abs @490nm

### Melanin induction

IBMX (3-Isobutyl-1-methylanxthine; Methylisobutylxanthine; MIX)

CAS 28822-58-4

sc-201188 (200mg, Santa Cruz)

MW 222.24

Dissolved in 1.112ml DMSO makes 1M stock sol'n.

Use 1:10000, ~48h culture

## Isolation of melanosomes from melanoma cell lines

Day 0

Plate Melanoma cell lines (B16 mouse melanoma, ~~SK-MEL-24 human melanoma~~)

@ $3 \times 10^5$ /10cm dish

(10cm dish x 1 for 1 or 2 assay(s) in 1~1.5ml cell culture)

Day 1

Add  $1/5000 \sim 10000$  IBMX

Day 6

Check if cells would become darken (~144h)

Collect cells by TE treatment followed by resusp w/ culture medium ~~scraper~~

↓

~~Pass through 25G syringe, 6 times (check breakage by Trypan blue staining) on ice~~

↓

Spin @100g, 5min (swing out)

Ppt.

↓

+PBS

↓

Spin @100g, 5min (swing out)

Ppt. +500 $\mu$ l PBS, susp

↓

-20oC until use

Thaw above

↓

+1ml lysis buffer\*, 5min, on ice

↓

Spin @300 x g, 3min, 4oC (swing out)

sup

↓

Spin @300 x g, 3min, 4oC (swing out)

sup

↓

Spin @max speed, 3min, 4oC (swing out)

Ppt

↓

wash w/ PBS x2

Ppt

↓

resusp in 0.1ml culture medium

Add to cells (~1ml culture) for uptake experiments

\*0.01% SDS, 1%Tx100, 0.1M Tris-HCl,