

Melanocyte Differentiation

Melanocytes growing in a proliferative medium such as CnT-40 tend to show a more fibroblastic phenotype, with fewer dendrites and lower melanin content.

Cells can be influenced to assume a more differentiated phenotype with increased melanin expression by switching to the CnT-PR-MD melanocyte differentiation medium. The following protocol is recommended for this procedure.

Important point: melanocytes differ in their ability to differentiate and produce melanin in vitro. Age in culture, and age of donor both have an effect. Young donors often show the highest responsiveness. Where possible, it is also advisable to compare a couple of different donors.

Induction of Differentiation

- 1) Seed in CnT-40 at usual density, and grow for 24 hours.
- 2) Switch to CnT-PR-MD, and grow for 5-7 days.
- 3) If whitening assays are to be conducted on the differentiated cells, it is recommended that the cells are exposed to the active ingredients over 4-5 days in CnT-PR-MH medium, to maximise cell responsiveness and assay sensitivity. Kojic acid (1.5 mM) may be used as a positive control.
- 4) Detach cells, perform cell counts, assay for total melanin (below), and then normalise the results by the cell number calculated previously.

Assaying for Total Melanin

There are a variety of methods for evaluating melanin content. One simple yet effective method for evaluating total melanin uses spectrophotometry as follows.

- 1) Detach cells with Accutase, and wash with PBS
- 2) Resuspended the cells in culture medium, count the cells and transfer the required number of cells for the assay into an eppendorf tube. Pellet the cells by centrifugation at 1500 rpm for 5 minutes
- 3) Aspirate supernatant, resuspend the pellet in 1 M NaOH, and incubate at 37°C for overnight
- 4) Centrifuge the tubes at 10000xg for 10 minutes at room temperature, and transfer 100µl of supernatant to a 96 well microplate
- 5) For standard curve: prepare 8 concs. of synthetic melanin by serial dilution from 500 µg/ml till 3.9 µg/ml. Transfer 100 µl of each diluted standard to a microplate in triplicates. Use NaOH as the blank.
- 6) Measure the absorbance of the supernatant at 405 nm
- 7) Normalise all ODs to the cell number
- 8) Plot a standard curve obtained from normalised synthetic melanin ODs (example: µg melanin/10⁴ cells)

For more information about your specific situation, please contact us via scientist@cellntec.com.