MELANIN QUANTIFICATION ASSAY

INTRODUCTION

Biobide is a biotechnology company offering discovery services to pharma, biotech, chemical and cosmetic companies. Our services are based on the **zebrafish** model and the capacity to offer highly efficient **tailor made assays**.

The zebrafish model is gaining relevance in due to its small size, transparency, ease manipulation and rapid development. This model has a high genetic homology with humans (over 85%), as well as an important parallelism in organogenesis and functional mechanisms.

The use of animals for cosmetic experimentation is banned, demanding other alternatives, in order to evaluate compound's safety and efficacy. The zebrafish model appears therefore, as an option for replacing superior animal experiments (1).

In cosmetic business, skin whitening is one of the most popular demands products, mainly for some Asiatic countries. Skin color is affected by melanin synthesis, which is produced in melanocytes in the basal layer of human epidermis. Melanin has important physiological function such as a major defense mechanism against visible and ultraviolet radiation.

In the last few years *Danio rerio* has appeared as an effective model to evaluate whitening capabilities (2). Zebrafish pigment pattern allows to study melanocyte development and melanin genesis.

METHOD DESCRIPTION

The protocol is divided on two main phases:

1. **Maximum Tolerated Concentration (MTC):** the MTC assay will give the information about the best range of concentrations to perform the efficacy assay.
2. **Melanin assay**: 24 hpf (hours post fertilization) embryos (n=100) will be dispensed in 20 mm plates and treated at the desired concentration with the test item/s. 100 µM PTU will be used as the bleached positive control and vehicle solvent as the negative control.

Data collection: after 24 hours of treatment, 48hpf embryos will be anesthetised on ice and washed with PBS.
Melanin content will be extracted by sonication in a PBS tween solution. Samples will be centrifuged and pellet dissolved in 1 ml of 1N NaOH. Samples will be heated at 100 °C for 20 minutes, subsequently vortexed and measured at 400 nm. A Melanin calibration curve will be used to calculate total sample melanin content.

The results will be expressed in mg/ml of melanin (Figure 1) with respect to vehicle control (Figure 2). Triplicated will be used for statistic analysis.

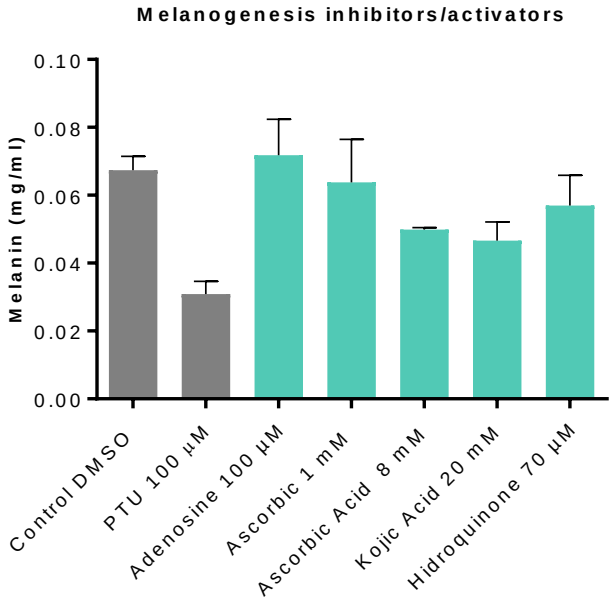

Figure 1. Effect of the compounds tested in melanin synthesis. Adenosine acts as a melanogenesis activator, while Ascorbic, Ascorbic Acid, Kojic Acid and Hidroquinone inhibit the melanin synthesis. DMSO was used as vehicle control and PTU 100 µM as positive melanogenesis inhibitor.

[1] Strähle U. 2012 Reprod Toxicol. Apr;33(2):128-32.
[2] Kil-Nam K. J.2015 Microbiol.Biotechnol. Apr, 25(4),448-451