ANTIOXIDATION 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 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, compounds with potential to reduce free-radical damage and environmental stress on the skin have become very popular. Skin is affected by ultraviolet radiation, environmental or aging factors, making necessary to find tools to defense it against them. To find antioxidant compounds with high efficacy is a major goal for Cosmetic Industry and have attracted a great deal of attention.

In the last few years *Danio Rerio* has appeared as an effective model to evaluate antioxidant capabilities (2). Zebrafish larvae allows to study the protective properties of cosmetic active ingredients against external factors.

Moreover, safety pharmacology screening, biochemical stability testing or *in vivo* cytotoxic performance testing can be done with this model.

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 anti-oxidative assay.
2. **Antioxidation assay**: for the assay (n=24), 4 days post fertilization (dpf) larvae will be dispensed independently in 96 plate well and treated at the desired concentration with the test item/s. Ascorbic acid 10 mM will be used as the antioxidant positive control and control vehicle as the negative control.

Data collection: after 24 hours of treatment, 5 dpf embryos will undergo to an oxidative treatment with H_2O_2 for 5 hours. After the oxidative treatment, CMFDA fluorescent dye will be used to detect the presence of thiol groups. CMFDA fluorescent dye, reacts with thiol groups utilizing a glutathione S-transferase-mediated reaction. After conjugation with Glutathion, CMFDA will be hydrolyzed to the fluorescent 5-chloromethyl-fluorescein by cellular esterases. The reaction's product can be detected by fluorescence using a wavelength 485/528.
At least 7 fluorescence measurements will be registered from each Larvae to determine the protective potential coming from the test item.

The results obtained after treatments with 3 different concentrations of L-Ascorbic are represented in Figure 1. Figure 2A presents a graph containing 17 measurements from an individual treated embryo with L-Ascorbic 10 mM, while Figure 2B presents a graph showing 17 measurements from a control larvae.

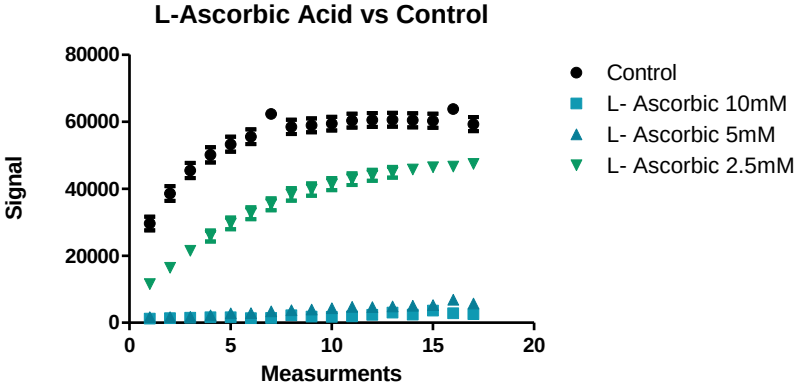

Figure 1. Average of 17 measurements obtained from 24 larvae showing the protective effect of 3 concentrations of L-Ascorbic vs un-treated controls larvae.

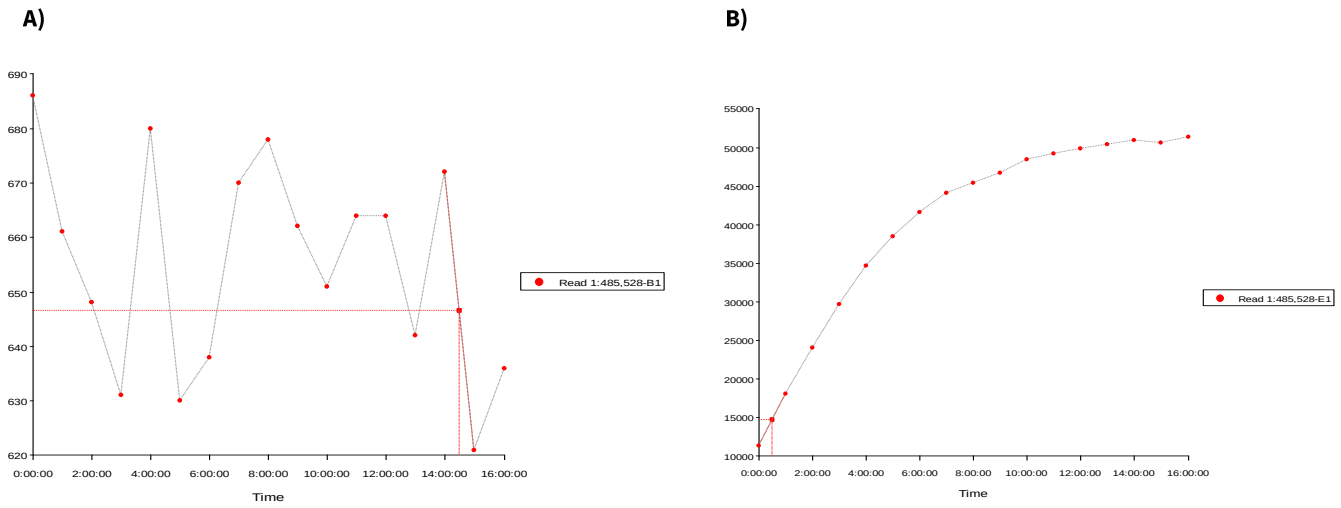

Figure 2. A) 17 measurements coming from an independent treated larvae with 10mM of L-Ascorbic. B) Signal obtained from a single un-treated larvae.

[1] Strähle U. 2012 Reprod Toxicol. Apr;33(2):128-32.
[2]Hu L. 2015 Acta Biochim Biophys Sin (Shanghai). 2015 May;47(5):349-54