

TEST CODE:
CT-023

Anti-Oxidant Activity Test

OXISELECT™ METHOD

OVERVIEW

Oxygen is an essential requirement for life, but it is also a very reactive molecule and can be harmful when converted to reactive oxygen species (ROS) within the body. ROS are unstable and react with most biological molecules such as DNA, proteins and lipids, contributing to inflammation and ageing. Stress and exposure to air pollutants can dramatically increase ROS levels in the skin and lead to an acceleration of the inflammatory and ageing pathways. Our cells are equipped with natural anti-oxidant enzymes to prevent ROS from forming, or to remove them before they cause damage. Providing our skin with an anti-oxidant boost through active ingredients in cosmetic products can help to enhance the skin's own natural protective mechanisms. Anti-oxidant ingredients may therefore support a variety of claims including anti-pollution and anti-ageing.

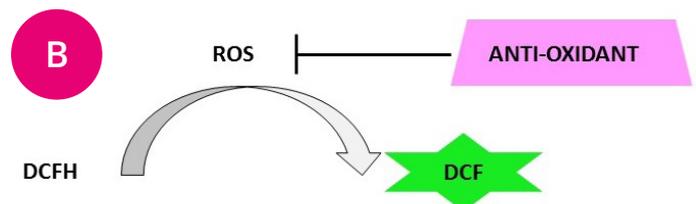
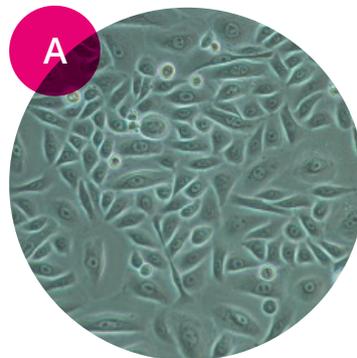
The OxiSelect™ test method exposes human skin cells (epidermal keratinocytes) to a range of concentrations of the active ingredient(s), either alone or in combination. Unlike anti-oxidant tests that utilise simple biochemical reactions in a test tube, the use of whole cells provides a more complete environment with higher physiological relevance, accounting for important aspects such as pH, cellular uptake and metabolism, as well as demonstrating the efficacy of the test item. OxiSelect™ captures anti-oxidant contributions from all major types of ROS, including peroxides, superoxides, hydroxyl radicals and singlet oxygen.

The test is based on the intracellular conversion of DCFH to its fluorescent metabolite (DCF). The reaction is inhibited by anti-oxidants, reducing the amount of fluorescence observed. OxiSelect™ is suitable for ingredients or formulations with sufficient aqueous solubility in cell culture medium.

TEST SYSTEM: HUMAN KERATINOCYTES IN ANIMAL-FREE CONDITIONS

A OxiSelect™ uses human skin cells (epidermal keratinocytes) in animal-product-free culture, providing a physiologically relevant environment. Cells are obtained from a commercial source with full donor consent and QC screening.

B The method uses a specialised marker called di-chloro-hydro-fluorescein diacetate (DCFH-DA), which diffuses into cells and is metabolised by cellular esterases. The metabolite (DCFH) is rapidly oxidised to highly fluorescent 2',7'-Di-chloro-hydro-fluorescein (DCF) by reactive oxygen species (ROS) in the presence of the proprietary "Free Radical Initiator". The fluorescence intensity is proportional to the levels of ROS within the cell, and is therefore reduced in the presence of anti-oxidant active ingredients. The plant flavonoid, quercetin, is used as a positive control.



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SUMMARY OF THE TEST METHOD

- Preliminary steps establish the solubility of the active ingredient, a cytotoxicity screen determines the highest concentration that the cells will tolerate without causing cell damage.
- Human epidermal keratinocytes are cultured until 80-90% confluent in 96-well plates, in animal product-free culture conditions.
- DCFH-DA probe solution is added to the cells along with Quercetin (positive control), or the active ingredient(s), at the specified concentrations.
- After incubation for 60 minutes at 37°C, cells are rinsed and Free Radical Initiator solution is added to the wells.
- Fluorescence intensity (to quantify DCF) is read immediately and monitored over a 60-minute period, in increments of 5 minutes.
- Results for each of the test conditions are plotted in graph format, to demonstrate any dose-dependent anti-oxidant potential of the active ingredients, either alone or in combination.

COSMETIC CLAIMS SUPPORTED

Anti-oxidant, Anti-pollution, Anti-ageing

TURNAROUND TIME

4 – 6 weeks

AMOUNT OF SAMPLE REQUIRED

Minimum 10ml (liquids) / 10g (solids)

PRICE

Our test prices are dependent on the quantity of test items. Please enquire for a quote using the contact information shown below, or the contact form on our website.

FURTHER DOWNLOADS

[XCellR8 Good Laboratory Practice \(GLP\) Compliance Certificate.](#)

QUALITY STATEMENT

XCellR8 is accredited by the UK Medicines and Healthcare Products Regulatory Authority (MHRA) for the conduct of *in vitro* safety testing in compliance with Good Laboratory Practice (GLP). This means that we are able to provide you with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The test method described here is non-regulatory but is conducted in our GLP-accredited laboratory.

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