TEST CODE: CT-XXX

ANTIOXIDANT ACTIVITY SCREENING

IN CHEMICO ASSESSMENT USING ABTS, CUPRAC, AND ORAC ASSAYS

OVERVIEW

Antioxidants are molecules that neutralise free radicals, unstable reactive species generated by UV light, pollution, metabolic processes, and other stressors that accelerate skin ageing. In cosmetics, antioxidants are essential because they help prevent oxidative damage to collagen, elastin, lipids, and DNA, thereby supporting firmer, brighter, and healthier-looking skin. They also reduce inflammation, strengthen the skin barrier, and improve the stability of cosmetic formulations by protecting sensitive ingredients from oxidation.

This test uses a battery of three complementary in chemico, cell-free assays to determine whether a test item exhibits intrinsic antioxidant activity through direct chemical radical scavenging and/or reducing capacity. The assays assess different antioxidant mechanisms: single electron transfer (SET) and hydrogen atom transfer (HAT) pathways.

Results are expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values in µM, allowing direct comparison of antioxidant strength against a Trolox standard. This battery approach reveals not only whether an ingredient has antioxidant activity, but also the dominant mechanism of action, which informs formulation strategy for anti-ageing and environmental defence products.

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TEST SYSTEM

THREE COMPLEMENTARY ASSAYS

ABTS ASSAY (MIXED SET AND HAT ACTIVITY)

The ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) measures the ability of the test item to quench the $ABTS^{\bullet+}$ radical cation, predominantly via single electron transfer (SET) with minor hydrogen atom transfer (HAT) contribution. This cation has a characteristic absorbance at 734 nm. When an antioxidant is present, the cation is reduced to its colourless form and a measurable decrease in absorbance proportional to the antioxidant strength occurs.

ORAC ASSAY (HAT ACTIVITY)

The ORAC assay (Oxygen Radical Absorbance Capacity) measures peroxyl radical scavenging capacity. This assay models chain-breaking antioxidant activity against biologically relevant peroxyl radicals generated from AAPH and is considered more physiologically relevant than purely electron-transfer assays, as peroxyl radicals are implicated in lipid oxidation and skin ageing. It is a hydrogen-atom transfer (HAT) assay in which peroxyl radicals are generated to slowly degrade a fluorescent probe. Antioxidants delay this fluorescence loss by neutralising the radicals. The instrument records this protection over time as an area-under-the-curve (AUC) value.

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TEST SYSTEM

SUMMARY OF THE TEST METHOD

Test items are typically evaluated at six concentrations spanning from the neat item to formulation-relevant concentrations, alongside a Trolox standard curve and vehicle controls.

ABTS ASSAY: The $ABTS^{\bullet+}$ radical cation is generated and exposed to test items. The reduction in absorbance at 734 nm is measured spectrophotometrically and compared to Trolox standards to calculate TEAC values.

CUPRAC ASSAY: Test items are incubated with cupric ions and neocuproine. The formation of the coloured Cu^{+}-neocuproine complex is measured spectrophotometrically at 450 nm. Results are expressed relative to Trolox standards as TEAC values.

ORAC ASSAY: A fluorescent probe is exposed to peroxyl radicals generated from AAPH in the presence of test items. Fluorescence decay is monitored over time. The area under the fluorescence decay curve is calculated and compared to Trolox standards to determine TEAC values.

All three assays are run concurrently to provide a comprehensive antioxidant profile, revealing whether the test item acts primarily through electron donation (SET-dominant), hydrogen donation (HAT-dominant), or a mixed mechanism.

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INTERPRETATION OF RESULTS

Results are expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in µM, representing the antioxidant activity of the test item relative to the Trolox standard.

Antioxidant Mechanism Classification:

- Low CUPRAC, moderate ABTS, high ORAC = HAT-dominant antioxidant
- High CUPRAC, moderate ABTS, low ORAC = SET-dominant antioxidant
- Balanced values across all three assays = Mixed mechanism antioxidant

Statistical significance is determined using two-tailed Student's t-test compared to vehicle controls. TEAC values can also be compared to industry benchmark ingredients to assess relative antioxidant performance.

TURNAROUND TIME
2 – 3 weeks

AMOUNT OF SAMPLE REQUIRED
5ml (liquids) / 5g (solids)
Please enquire if sample availability is limited.

QUALITY STATEMENT

This is a non-regulatory test designed for cosmetic ingredient screening and product development. 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).

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