TEST CODE: CT-102

Collagen Boosting Test

SOLUBLE COLLAGEN ASSESSMENT: SIRIUS RED METHOD

OVERVIEW

Collagen is the primary structural protein of the skin, responsible for its tensile strength, elasticity, and firmness. As skin ages, the synthesis of collagen by dermal fibroblasts decreases, leading to visible signs of ageing such as wrinkles, sagging, and loss of skin tone. This degradation is accelerated by extrinsic factors like UV exposure, which triggers matrix metalloproteinases (MMPs) that further break down existing collagen fibres.

The ability of a cosmetic ingredient to stimulate collagen production is a key indicator of its anti-ageing potential. This test directly measures the concentration of soluble collagen secreted by human dermal fibroblasts following exposure to test items, providing quantitative evidence to support collagen-boosting claims.

This test uses the Sirius Red method, in which Sirius Red dye selectively binds to the helical structure of soluble collagen. The collagen-dye complex is measured spectrophotometrically, and results are expressed as collagen concentration (µg/mL) and fold-change or percentage change relative to untreated control cells.

XCellR8
Redefining testing

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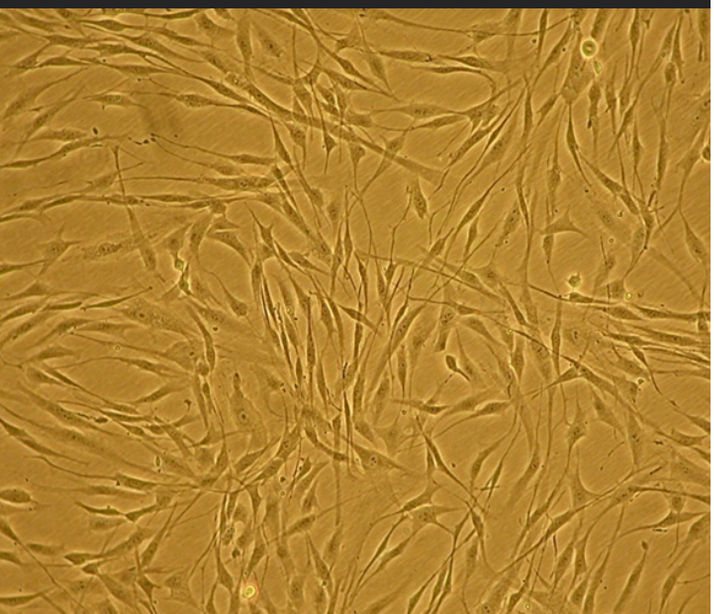
TEST SYSTEM

HUMAN DERMAL FIBROBLASTS

Adult human dermal fibroblasts (HDFa) are primary cells cultured from donor tissue under xeno-free conditions (no animal-derived components). Fibroblasts are the natural collagen-producing cells of the dermis and are widely used as a physiologically relevant model for assessing anti-ageing ingredients.

Cells are cultured to confluency in fibroblast growth medium containing human serum, then switched to serum-free conditions during test item exposure to isolate the effect of the test item on collagen production. This approach eliminates background collagen from serum and ensures that any measured collagen is newly synthesised and secreted by the fibroblasts in response to treatment.

The MTT test is used to assess cell viability, ensuring that collagen increases are due to enhanced production rather than cytotoxic effects.



TEST SYSTEM

SUMMARY OF THE TEST METHOD

Human dermal fibroblasts are seeded into multi-well plates and cultured to confluency. Culture medium is then replaced with serum-free medium containing the test item at multiple concentrations (typically determined by preliminary cytotoxicity assessment). A vehicle control and a positive control (Vitamin C) are included in parallel.

Cells are incubated for 24 to 48 hours, during which collagen is synthesised and secreted into the culture medium. At defined timepoints (typically 24 hours and 48 hours), culture medium is collected and the concentration of soluble collagen is quantified using the Sirius Red test.

The Sirius Red test uses a specially formulated dye reagent, which binds specifically to the glycine-X-Y helical structure of soluble collagen under controlled test conditions. Following dye binding, the collagen-dye complex is precipitated by centrifugation, washed, and the dye is released using an alkaline solution. The absorbance of the released dye is measured spectrophotometrically at 530-560 nm and compared to a collagen standard curve to calculate collagen concentration.

Cell viability is assessed using the MTT test. For samples showing cytotoxicity, collagen concentration can be normalised to the surviving cell fraction to account for reduced cell number.

Results are expressed as collagen concentration (µg/mL), fold-change and / or percentage change relative to the vehicle control, and statistical significance is determined using Student's t-test.

INTERPRETATION OF RESULTS

Results are presented as the concentration of soluble collagen (µg/mL) detected in the culture medium, along with fold-change and / or percentage change relative to untreated control cells. Positive results indicate that the test item stimulates fibroblasts to produce and secrete more collagen *in vitro* than baseline levels.

Dose-dependent responses (increasing collagen with increasing concentration) provide stronger evidence of a genuine collagen-boosting effect. Time-dependent responses (greater collagen accumulation at 48 hours than 24 hours) demonstrate sustained production over the exposure period.

Statistical significance is assessed using two-tailed Student's t-test. Vitamin C is included as a positive control to validate test performance, as it is a well-established cofactor for collagen synthesis.

Results can be compared to industry benchmark ingredients to assess relative collagen-boosting performance.

TURNAROUND TIME
2 – 3 weeks

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

PRICE

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

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).