

TEST CODE:
CT-038

Skin Sensitisation Test

KERATINOSENS™ (OECD TEST GUIDELINE 442D)

OVERVIEW

Skin sensitisation is defined as an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

The test described here is fully accepted at a regulatory level for the hazard identification of skin sensitisers and non-sensitisers in accordance with the UN GHS. It is appropriate for compliance with a range of legislation including the EU Cosmetics Regulation 1223/2009 and the CLP Regulation 1272/2008. It was incorporated into REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) legislation in 2016. Data generated using the KeratinoSens™ method should be considered in the context of an integrated approach i.e. in combination with other *in vitro* and *in chemico* skin sensitisation models.

The adverse outcome pathway leading to skin sensitisation involves a number of key events. This test method addresses Key Event 2, which takes place in the cells of the epidermis (keratinocytes). It induces inflammatory responses as well as gene expression associated with specific cell signalling pathways. The KeratinoSens™ cell line is a human keratinocyte cell line, containing a luciferase gene that is linked with a gene known to be up-regulated by contact sensitisers. In this test, a sensitising test item switches on the luciferase-linked gene, meaning that luciferase itself is also produced by the cells. Quantitative measurement (by luminescence detection) of luciferase activity is then carried out using a standard substrate. The prediction model correlates the luciferase signal with sensitisation potential. A test item inducing luciferase activity by more than or equal to 1.5-fold over the untreated control level is classified as Positive (Sensitiser). A test item causing less than 1.5-fold induction is classified as Negative (Non-Sensitiser).

The standard KeratinoSens™ test includes the use of animal-derived components in the test system, such as bovine serum in the cell culture medium. In order to provide our clients with a truly animal-free test, XCellR8 has adapted the test to animal product-free conditions, and validated the adapted method using the OECD listed proficiency chemicals and Performance Standards. This work has been published in the peer-reviewed literature (see Further Downloads on page 2). In 2016, the European Chemicals Agency (ECHA) confirmed that the adapted method can be used in REACH registration dossiers, and the protocol has since been published in the new edition of OECD Test Guideline 442D (adopted June 2018).

TEST SYSTEM: KERATINOSENS™ CELL LINE

The KeratinoSens™ cell line was created from an immortalized human keratinocyte line called HaCaT. The cells were transfected with a plasmid containing the luciferase gene under the transcriptional control of an Antioxidant Response Element (ARE) from a gene that is known to be up-regulated by contact sensitisers. Luciferases are a group of oxidating enzymes that convert marker substrates into light-emitting products that can be quantified by luminescence. The luciferase activity signal measured in the test reflects the activation by sensitisers of endogenous Nrf2 dependent genes in the presence of sensitising test items. KeratinoSens™ cells must be maintained under strictly controlled culture conditions to maintain expression of the luciferase gene.



XCellR8 Ltd. +44 (0)1925 607 134 | info@x-celler8.com | www.x-celler8.com
Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire, WA4 4AB, UK
Registered in England and Wales 6489519 | VAT number GB 932 3310 59.

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SUMMARY OF THE TEST METHOD

- KeratinoSens™ cells are seeded into 96-well plates and cultured at 37°C / 5% CO₂ for 24 hours.
- On the day of testing, test items are prepared as stock solutions in DMSO and serially diluted in culture medium to give 12 test concentrations ranging from 0.098mM to 200mM. The final concentration of DMSO in the test plate wells is 1%.
- The negative control is 1% DMSO and the positive control is cinnamic aldehyde.
- For each test item, 3 independent runs of the test are performed, each consisting of 3 replicate plates. Therefore the total number of data points used for statistical analysis is 9.
- The dosed plates are incubated at 37°C / 5% CO₂ for 48 hours.
- At the end of the exposure period, the plates are washed with phosphate buffered saline, prior to the addition of lysis buffer to each well for 20 minutes at room temperature.
- Plates are then placed into a validated luminometer, which is programmed to add the luciferase substrate (50µl), wait for 1 second, then integrate luciferase activity for 2 seconds.
- A cytotoxicity test (the MTT method) is performed in parallel plates, ensuring that the prediction of sensitisation potential is made using healthy, viable cells.
- Data is analysed to provide the following parameters:
 - The EC1.5 value is the lowest concentration of test item that causes a luciferase induction greater than 1.5-fold above the negative control. If any of the test concentrations exceed or equal the 1.5 threshold, the test item is classified as Positive (Sensitiser). If no test concentrations exceed the 1.5 threshold, the test item is classified as Negative (Non-Sensitiser).
 - The I_{MAX} value is the maximum induction achieved at any of the test concentrations. For example, an I_{MAX} value of 3 means that the maximum-fold induction observed at any test concentration was 3. Although the KeratinoSens™ test is not validated to predict potency, the I_{MAX} value can provide a useful tool for a very preliminary comparison of sensitisation potential between test items. For reference, during test validation, sensitising Proficiency Chemicals produced I_{MAX} values of up to 26.
- A range of acceptance criteria must be satisfied in order for each of the 3 independent experimental runs, and the overall test, to be considered valid.

TURNAROUND TIME

6 – 8 weeks

AMOUNT OF SAMPLE REQUIRED

10ml (liquids) / 10g (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 below, or the contact form on our website.

FURTHER DOWNLOADS

[OECD Test Guideline 442d](#)
[XCellR8 Good Laboratory Practice \(GLP\) Compliance Certificate.](#)
[Peer Reviewed Paper \(ALTEX Journal 2017\).](#)

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 our clients with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The regulatory status is applicable in all global territories participating in the OECD's Mutual Acceptance of Data.

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