TEST CODE:
CT-039

Skin Sensitisation Test

DIRECT PEPTIDE REACTIVITY ASSAY (DPRA) (OECD TEST GUIDELINE 442C)

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) in 2016. Data generated using the DPRA 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 the molecular initiating event (Key Event 1) – reactivity with proteins – by quantifying the reactivity of test items towards model synthetic peptides containing either cysteine or lysine. The DPRA is an *in chemico* method which quantifies the remaining concentrations of cysteine- and / or lysine-containing peptide following 24 hours incubation with the test item at 25+/-2.5°C. Relative peptide concentration is measured by High Performance Liquid Chromatography (HPLC) with gradient elution and UV detection at 220nm. Cysteine and lysine percentage peptide depletion values are then used to categorise a test item in one of four classes of reactivity (Minimal, Low, Moderate or High). For hazard identification purposes, test items in the Minimal reactivity class are classified as Negative (Non-Sensitisers). Test items in the Low, Moderate or High reactivity classes are classified as Positive (Sensitisers).

TEST SYSTEM:

The test system consists of an *in chemico* incubation of the test item with defined synthetic peptides containing cysteine (Ac-RFAACAA-COOH) and lysine (Ac-RFAAKAA-COOH). The purity of the synthetic peptides must be >85% and they must be freshly prepared immediately prior to incubation with the test item.

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XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

- Stock solutions of cysteine and lysine containing synthetic peptides of >85% purity are freshly prepared just prior to incubation with the test item. The final concentration of the cysteine peptide is 0.667mM in pH7.5 phosphate buffer and the final concentration of the lysine peptide is 0.667mM in pH10.2 ammonium acetate buffer.
- Solubility of the test item in an appropriate solvent (as described in OECD Test Guideline 442c) is assessed in advance and stock solutions of 100mM concentration are prepared just before the test.
- The positive control is 100mM cinnamic aldehyde in acetonitrile. Reference controls (containing only the peptide in the appropriate solvent) are also included and are used to calculate the percentage peptide depletion for each test item. In addition, a co-elution control (containing only the test item in the appropriate solvent) is included in the HPLC run to detect possible co-elution of the test item with either the cysteine or lysine peptide.
- Cysteine and lysine peptide solutions are incubated in glass autosampler vials with the test item at 1:10 and 1:50 ratio respectively. The reaction solution is left in the dark at 25±2.5°C for 24±2 hours before running the HPLC analysis.
- A standard calibration curve is generated for both the cysteine and lysine peptides. Six standards are prepared using serial dilutions of the 0.667mM stock solution, to cover the final concentration range from 0.534mM to 0.0167mM. A blank of the dilution buffer is also included and calibration curves must have an r^2>0.99.
- Peptide depletion is monitored by High Performance Liquid Chromatography (HPLC), using a reversed-phase column, gradient elution and UV detection at 220nm.
- The percentage peptide depletion is determined by measuring the peak area and dividing it by the mean peak area of the relevant reference controls.
- A range of acceptance criteria must be satisfied in order for the test to be considered valid.

TURNAROUND TIME	*8 – 12 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 442c 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 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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