TEST CODE:
CT-043

Skin Sensitisation Test

HUMAN CELL LINE ACTIVATION TEST (h-CLAT) (OECD TEST GUIDELINE 442E)

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 supports discrimination of skin Sensitisers and Non-Sensitisers in accordance with the UN GHS. The h-CLAT test also provides a useful screen for product development. Data generated using the h-CLAT 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.

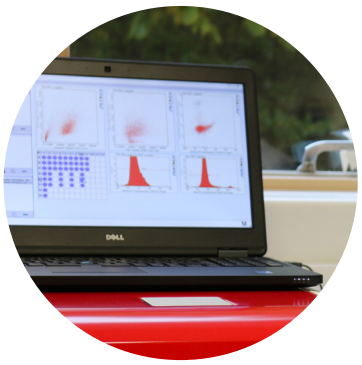
The adverse outcome pathway leading to skin sensitisation involves a number of key events. This test method addresses Key Event 3 – activation of Dendritic Cells (DC). Skin sensitisers have been reported to induce the expression of cell membrane markers (CD54 and CD86) associated with DC activation. These surface molecules are typical markers of DC activation that play a critical role in T-cell priming by DCs, activating an immune response.

The h-CLAT method is an *in vitro* test that quantifies changes of cell surface marker expression (CD54 and CD86) following 24 hour exposure to a test item using a human monocytic leukaemia cell line (THP-1) that mimics DC activation. The changes of surface marker expression are measured by flow cytometry following cell staining with fluorochrome-tagged antibodies. Cytotoxicity measurement is also conducted concurrently to assess whether upregulation of surface marker expression occurs at sub-cytotoxic concentrations. The relative fluorescence intensity of surface markers compared to solvent/vehicle controls are calculated and used in the prediction model to support the discrimination between Sensitisers (positive for cell surface marker up-regulation) and Non-Sensitisers (negative for cell surface marker upregulation).

The standard h-CLAT test includes the use of animal-derived components in the test system, such as serum and antibodies. In order to provide our clients with a truly animal-free test, XCellR8 has adapted the test to animal product-free conditions. Proficiency testing as per OECD Test Guideline 442e has demonstrated our successful classification of a panel of reference chemicals in terms of skin sensitisation potential. This work has been published in the peer-reviewed literature (see Further Downloads on page 2). The adapted test is suitable for submissions under REACH and, following discussion at the OECD Expert Working Group on skin sensitisation, is currently under consideration by the WNT (National Co-ordinators' Committee) for listing in OECD TG 442e. A full technical report on the Proficiency Testing and further regulatory details are available on request.

TEST SYSTEM

The test system consists of THP-1 cells that are incubated *in vitro* with a test item for 24 hours followed by detection of fluorochrome-tagged anti-CD54 and anti-CD86 antibodies by flow cytometry.



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XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

Dose Finding Assay (CV75)

- THP-1 cells are pre-cultured for either 48 or 72 hours
- At the end of the THP-1 pre-culture period, a stock solution of the test item is prepared in an appropriate solvent and diluted over an 8 dose range
- Vehicle (solvent and positive) controls are prepared
- The diluted test item and the vehicle control are incubated with THP-1 cells in a 96 well plate for 24 hours
- After the 24 hour exposure period, THP-1 cells are washed and then stained with propidium iodide
- Propidium iodide is taken up into dead cells and can therefore be used to separate the live cell population from the dead cell population
- The % of live cells is determined using flow cytometry and the data used to derive the test item concentration at which 75% of the cells are live (CV75)
- A range of acceptance criteria must be satisfied in order for the test to be considered valid
- Two independent runs are required to derive the CV75

Measurement of CD54 and CD86 expression

- THP-1 cells are pre-cultured for either 48 or 72 hours
- At the end of the THP-1 pre-culture period, a stock solution of the test item is prepared in the previously determined solvent and diluted over an 8 dose range around the CV75 value
- Vehicle (solvent) controls and a positive control that is known to increase expression of CD54 and CD86 are prepared
- The diluted test item, the vehicle control and the positive control are incubated with THP-1 cells in a 24 well plate for 24 hours
- After the 24 hour exposure period, THP-1 cells are washed and then stained with propidium iodide and either the anti-CD54, anti-CD86 or negative control antibodies
- Expression of CD54 and CD86 is measured in 10,000 live cells from each test condition using flow cytometry
- The THP-1 cell expression of CD54 and CD86 after exposure to the test item is calculated relative to the vehicle control
- If the expression level crosses the pre-set threshold for one or both of the cell surface markers at one of the test item concentrations where the cell viability is above 50% the test item is considered to be positive i.e. a sensitiser
- A range of acceptance criteria must be satisfied in order for the test to be considered valid
- Two concordant independent runs are required to derive the final h-CLAT prediction

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 442e XCellR8 Good Laboratory Practice (GLP) Compliance Certificate. Peer Reviewed Paper (ALTEX Journal 2018)

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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