TEST CODE:
CT-050

3D Phototoxicity Test

RECONSTRUCTED HUMAN SKIN MODEL METHOD

OVERVIEW

Phototoxicity, or photo-irritation, is an acute toxic response following exposure of skin to certain chemicals and subsequent exposure to light. It can also be induced by skin irradiation after systemic administration of a chemical substance.

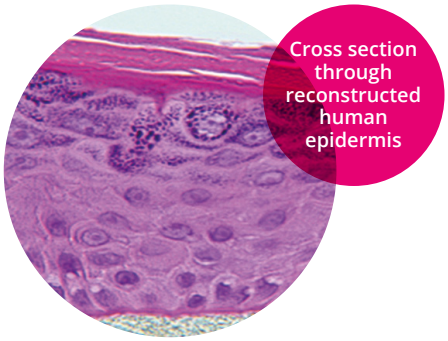
The test described here is a non-regulatory method that detects the phototoxic potential of a chemical, by using a human reconstructed epidermis. The model is grown on special platforms at the air-liquid interface, allowing for direct application of test items in a way that accurately models "real life" skin exposure.

Test items are applied onto the skin surface and exposed to UVA radiation. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from skin tissues. Phototoxic potential is calculated by comparing to tissues not exposed to UVA, determined one day after chemical treatment and UVA exposure.

TEST SYSTEM: RECONSTRUCTED HUMAN EPIDERMIS

Reconstructed human epidermis is a skin model composed of living human keratinocytes which have been cultured to form a multi-layered, highly differentiated epidermis. The levels of differentiation obtained are at the cutting edge of *in vitro* skin technology. The model consists of highly organized basal cells which progressively flatten out as the apical surface of the tissue is approached, analogous to the normal human *in vivo* epidermis. The model includes a functional skin barrier with an *in vivo*-like lipid profile. The profiles of key differentiation markers also mirror those seen *in vivo*. The cells are both metabolically and mitotically active.

Since the test allows application of test materials to the air exposed skin surface (*stratum corneum*), it can be used to assess the phototoxic potential of either ingredients or final formulations, irrespective of their solubility in aqueous cell culture medium.



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CT-050/01-03/18

XCellR8 Redefining testing

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3D Phototoxicity Test

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SUMMARY OF THE TEST METHOD

- Skin models are pre-incubated in a cell culture incubator (37°C/5% CO2) for 60 minutes or overnight.
- Five concentrations of the test item are applied to the tissue surface (50µl for water soluble compounds or 25µl for oil-soluble compounds).
- Controls consist of solvent water or oil (negative control) and 5 concentrations of chloropromazine (positive control).
- The dosed skin models are placed in a cell culture incubator overnight, using at least duplicate samples for each concentration.
- After overnight incubation, one set of tissues is exposed to a defined dose of UVA, while a second set is left in the dark.
- Test items and control substances are removed from the skin model surface by washing.
- Tissues are incubated overnight in a cell culture incubator (37°C/5% CO2).
- The viability of the skin models is assessed by MTT conversion. MTT solution is applied at the basal side of the tissues, which are placed into a cell incubator for 3 hours. The blue formazan metabolite produced by viable cells is then extracted into isopropanol by incubation at room temperature for 2 hours or overnight.
- Duplicate samples of the extracted formazan solution are transferred to a microplate and the formazan product is quantified by absorbance spectrophotometry (wavelength 570nm).
- Absorbance readings of the formazan product from skin models incubated with test items and the positive control are used to calculate percentage viability, relative to the negative control.
- Identical calculations are performed for the (+UVA) part of the test and the (-UVA) part of the test.
- A range of acceptance criteria must be satisfied for the experimental run to be declared valid.
- A prediction model is used to determine the phototoxic potential of the test item, which is predicted to have a phototoxic potential if one or more test concentrations of the (+UVA) part of the experiment reveal a decrease in viability exceeding 30% when compared with identical concentrations of the (-UVA) part of the experiment.
- Prediction of phototoxicity is supported if, in addition, the (+UVA) induced reduction in tissue viability shows a dose response relationship.

TURNAROUND TIME	*6 – 8 weeks*
AMOUNT OF SAMPLE REQUIRED	*Minimum 10ml (liquids) / 10g (solids)*
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.*

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 you with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The test method described here is non-regulatory but is conducted in our GLP-accredited laboratory.

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