



In vitro Skin Sensitization

A skin sensitizer refers to a substance that will lead to an allergic response following skin contact as defined by the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals GHS.

For any chemical likely to be in contact with human skin must be assessed for its skin sensitising potential. The key chemical and biological events associated with skin sensitisation are summarised in adverse outcome pathway (AOP), starting from molecular initiating event through intermediate events to the adverse effect, namely allergic contact dermatitis.

JRF offers three tiered *in vitro* skin sensitisation tests based on AOP principle and an alternative to replace the current prevalent *in vivo* methods.

- Direct Peptide Reactivity Assay (DPRA) (OECD 442C)
- ARE-Nrf2 Luciferase Test (KeratioSens) (OECD 442D)
- Human Cell Line Activation Test (hCLAT) Myeloid U937 Skin Sensitization Test (MUSST/U-SENS) (OECD 442E)

Direct Peptide Reactivity Assay (DPRA) (OECD 442C)

It evaluates potential sensitisation as a result of depletion of the specific synthetic hepta-peptides of lysine & cysteine. Haptenisation, i.e., the covalent binding of low-molecular weight substances (haptens) to proteins in the skin, is considered as a prominent mechanism, through which drugs or their metabolites could become antigenic. Therefore, information from peptide reactivity assays such as the DPR Assay is considered relevant for the assessment of the skin sensitisation potential.

ARE-Nrf2 Luciferase Test (KeratinoSens)[™] (OECD442D)

is based on the keratinocyte activation of an immortalised adherent cell line, derived from HaCaT human keratinocytes, stably transfected with a selectable plasmid. The cell line contains the luciferase gene, under the transcriptional control of the SV40 promoter fused with an ARE element from the AKR1C2 gene which is known to be up-regulated by contact sensitisers. The Luciferase signal is measured, using a Chemiluminescence measuring device. This allows quantitative measurement of luciferase gene induction, using well established light producing luciferase substrates, as an indicator of the activity of the Nrf2 transcription factor in cells following exposure to electrophilic test substances.

Human Cell Line Activation Test (h-CLAT) (OECD442E)

is an *in vitro* assay that quantifies changes of cell surface marker expression (i.e., CD86 and CD54) on a human monocytic leukemia cell line, THP - 1, following 24 hours exposure to the test chemical. These surface molecules are typical markers of monocytic THP- 1 activation and may mimic Dendritic cell (DC) activation, which plays a critical role in T - cell priming. The changes of surface marker expression are measured by flow cytometer, following cell staining with fluorochrome - tagged antibodies. Cytotoxicity measurement is also conducted concurrently to assess whether up-regulation of surface marker expression occurs at sub - cytotoxic concentrations. The relative fluorescence intensity of surface markers, compared to solvent / vehicle control, are calculated and used in the prediction model, to support the discrimination between sensitisers and non - sensitisers.

