cyprotex AN EVOTEC COMPANY

Skin and Ocular Testing

KeratinoSens™ Skin Sensitisation Assay

Background Information

'Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by e.g., covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes'

[4]OECD Guideline for the Testing of Chemicals. Draft Proposal for a New Test Guideline: *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, May 2014

- The Keap1-Nrf2-ARE pathways have been shown to be a major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitisation.[1,2,3]
- The KeratinoSens™ assay uses an immortalised adherent human keratinocyte cell line (HaCaT cell line), transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE)[1] and has been validated as a useful *in vitro* system for assessing the skin sensitising potential of compounds.
- In February 2014, KeratinoSens™ was recommended by EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) for use within an integrated strategy for skin sensitisation testing. An OECD test guideline (OECD TG 442D) was released in February 2015.[4]
- Cyprotex offer the KeratinoSens™ assay in accordance with the OECD test guideline.

Protocol

Cell Line
KeratinoSens™ cell line

Analysis
Induction of luciferase reporter gene expression and cell viability

Test Article Concentrations
12 concentrations in triplicate in 3 independently performed experiments

Highest concentration 2000 µM (according to OECD guideline, dependent on customer requirements)

Time Points
48 hrs

Quality Controls
Vehicle control: 1% DMSO (vehicle)
Positive control: cinnamic aldehyde
Negative control: sodium dodecyl sulfate

Data Delivery
Dose response curves for cell viability and luciferase reporter gene expression

MEC (minimum effective concentration), AC_{50}, IC_{30} and maximum response (%) for cell viability

$EC_{1.5}$, AC_{50} and maximum response (I_{max}) for luciferase gene expression as well as sensitising potential classification

'The KeratinoSens™ test method was considered scientifically valid to be used as part of an IATA, to support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling.'[4]

Table 1

Data from the KeratinoSens™ assay for 13 compounds with comparison to literature data including the OECD draft guidelines.[4,5,6]

		Literature Data[4,5,6]			Cyprotex Data		
Compound	*In vivo*	Classification	$EC_{1.5}$* (µM)	IC_{50} (µM)	Classification	$EC_{1.5}$* (µM)	IC_{50} (µM)
Non-sensitising compounds							
Isopropanol	Non-sensitiser	Negative	>1000	>1000	Negative	>1000	>1000
Salicylic acid	Non-sensitiser	Negative	>1000	>1000	Negative	>1000	>1000
Lactic acid	Non-sensitiser	Negative	>1000	>1000	Negative	>1000	>1000
Glycerol	Non-sensitiser	Negative	>1000	>1000	Negative	>1000	>1000
Sodium dodecyl sulphate	Non-sensitiser	Negative	NR	NR	Negative	>31	54
Sensitising compounds							
Cinnamyl alcohol	Weak	Positive	25-175	>1000	Positive	119	>1000
Ethylene glycol dimethacrylate	Weak	Positive	5-125	>500	Positive	39	810
2-Mercaptobenzothiazole	Moderate	Positive	50-250	>500	Positive	1187	1025
Methyldibromo glutaronitrile	Strong	Positive	<20	20-100	Positive	9.1	25
4-Methylaminophenol	Strong	Positive	<12.5	20-200	Positive	3.3	15
2,4-Dinitro-1-chlorobenzene	Extreme	Positive	<12.5	5-20	Positive	1.5	8
Cinnamic aldehyde	Weak	Positive	NR	NR	Positive	13	100
2,3-Butanedione	Weak	Positive	<100	NR	Positive	54	370

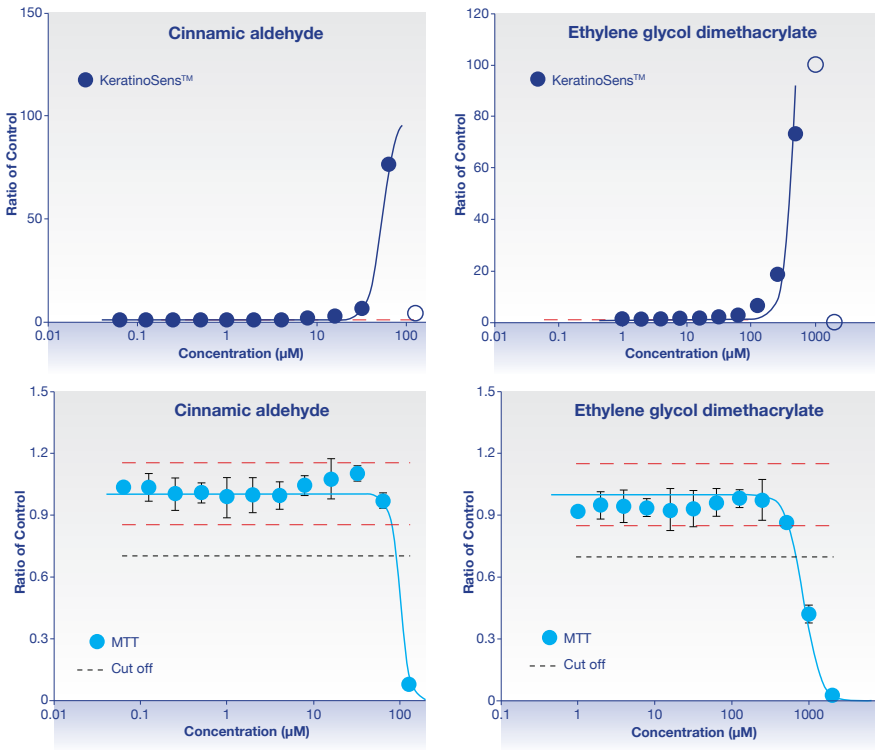
NR = not reported

* $EC_{1.5}$ represents the concentration for which gene induction is above the 1.5-fold threshold (i.e., 50% enhanced gene activity is obtained)

The KeratinoSens™ data illustrated in Table 1 comprises of eight sensitising compounds and five non-sensitising compounds. All compounds were predicted correctly when compared with the *in vivo* classification, with previously published data and the proficiency compounds of the OECD draft guidelines.

Figure 1

Data from the KeratinoSens™ assay for the skin sensitisers cinnamic aldehyde and ethylene glycol dimethacrylate. The upper graphs illustrate the activation of the luciferase reporter with increasing concentrations of test article. The lower graphs illustrate MTT data which are used to assess cytotoxicity of the test article. The points on the upper graphs are excluded if they exceed the cytotoxicity limit and these points are illustrated as open blue circles.



References

1 Emter R *et al.*, (2010) Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers *in vitro*. *Toxicol Appl Pharmacol* **245(3)**; 281-290
2 Natsch A *et al.*, (2010) The Nrf2-Keap1-ARE toxicity pathway as a cellular sensor for skin sensitizers – Functional relevance and hypothesis on innate reactions to skin sensitizers. *Toxicol Sci* **113(2)**; 284-292
3 Dinkova-Kostova AT *et al.*, (2005) The role of Keap1 in cellular protective responses. *Chem Res Toxicol* **18(12)**; 1779-1791
4 OECD Guideline for the Testing of Chemicals. *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, Adopted February 2015
5 Bauch C *et al.*, (2012) Putting the parts together: Combining *in vitro* methods to test for skin sensitizing potentials. *Regul Toxicol Pharmacol* **63(3)**; 489-504
6 Natsch A *et al.*, (2011) The intra- and inter-laboratory reproducibility and predictivity of the KeratinoSens assay to predict skin sensitizers *in vitro*: Results of a ring-study in five laboratories. *Toxicol In Vitro* **25(3)**; 733-744