Skin and Ocular Testing

KeratinoSens™
Skin Sensitisation Assay

Background Information

- 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\(_{\text{max}}\)) for luciferase gene expression as well as sensitising potential classification

To find out more contact enquiries@cyprotex.com
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.

Table 1
Data from the KeratinoSens™ assay for 13 compounds with comparison to literature data including the OECD draft guidelines.4,5,6

<table>
<thead>
<tr>
<th>Compound</th>
<th>In vivo Classification</th>
<th>IC50 IC1.5 (µM) Cyprotex Data</th>
<th>In vivo Classification</th>
<th>IC50 IC1.5 (µM) Cyprotex Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sensitising compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopropanol</td>
<td>Negative</td>
<td>NR</td>
<td>Negative</td>
<td>NR</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>Positive</td>
<td>25-175</td>
<td>Positive</td>
<td>119</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Positive</td>
<td>5-125</td>
<td>Positive</td>
<td>39</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Weak</td>
<td>0.9</td>
<td>Positive</td>
<td>25</td>
</tr>
<tr>
<td>Sodium dioctyl sulphate</td>
<td>Positive</td>
<td>20</td>
<td>Positive</td>
<td>0.6</td>
</tr>
<tr>
<td>Sensitising compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>Weak</td>
<td>0.6</td>
<td>Positive</td>
<td>0.9</td>
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<tr>
<td>Ethylene glycol dimethacrylate</td>
<td>Strong</td>
<td>0.4</td>
<td>Positive</td>
<td>0.7</td>
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<tr>
<td>2-Mercaptobenzothiazole</td>
<td>Weak</td>
<td>2.5</td>
<td>Positive</td>
<td>2.5</td>
</tr>
<tr>
<td>Methylthiomethyl glutaricnitrile</td>
<td>Strong</td>
<td>1.5</td>
<td>Positive</td>
<td>1.5</td>
</tr>
<tr>
<td>4-Methylinchophenol</td>
<td>Weak</td>
<td>1.5</td>
<td>Positive</td>
<td>1.5</td>
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<tr>
<td>2,4-Dinitro-1-chlorobenzene</td>
<td>Extreme</td>
<td>1.5</td>
<td>Positive</td>
<td>1.5</td>
</tr>
<tr>
<td>Cinnamyl aldehyde</td>
<td>Weak</td>
<td>1.5</td>
<td>Positive</td>
<td>1.5</td>
</tr>
<tr>
<td>2,3-Butanedione</td>
<td>Weak</td>
<td>1.5</td>
<td>Positive</td>
<td>1.5</td>
</tr>
</tbody>
</table>

NR = not reported

* EC1.5 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.

References
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
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(2); 733-744