**Abstract**

To reduce time, expenses and resources in drug discovery, in vitro toxicity assays are used to detect adverse drug effects in earlier phases of drug development. In recent years, in vitro studies with primary human isolated hepatocytes have replaced animal models as the gold standard for liver toxicity studies, as they provide more accurate and less costly testing than animal models. However, primary human hepatocytes are available in limited supply and can exhibit variability between donor samples. Although cell lines, such as HepG2 cells, have been used to overcome these limitations, HepG2 cells display expression of drug-metabolizing enzymes that significantly differs from primary hepatocytes. Corning® HepatoCells are immortalized cryopreserved cells, derived from primary human hepatocytes that are consistent lot-to-lot and display primary hepatocyte characteristics, such as robust CYP3A4, 1A2, and 2B6 fold induction similar to primary hepatocytes. In conventional in vitro cytotoxicity assays, specific cytotoxic indicators, such as ATP depletion, must be measured, but the mechanisms of chemical injury can be diverse, and therefore several different indicators must be monitored. Additionally, the use of fluorescent or luminescent dyes, or radiolabeled compounds can introduce variables into the assay that are not replicated in vivo. Label-free dynamic mass redistribution (DMR) assays using Corning Epic technology offer an alternative approach, capturing the cell signaling activity that occurs upon drug exposure in a single-time-resolved measurement. In this study, primary hepatocytes, HepG2 cells, and HepatoCells were assayed with known hepatotoxins using the Corning Epic BT reader, demonstrating the ability of the technology to detect cytotoxic responses, and comparing the responses between the three cell types.

**Toxicity Assays with Corning Epic Technology**

**Bioactivation to Reactive Metabolites – Tacrine**

All three cell types displayed a dose-dependent N-DMR response to Tacrine. Dose responses are plotted as AUC. Corning HepatoCells displayed the largest response in magnitude to Tacrine.

**Negative Control – Menthol**

All three cell types failed to display dose-dependent responses to the negative control compound menthol. Dose responses are plotted as AUC. This data supports that the previously displayed toxic responses were specific.

**Table of TC50 Values**

<table>
<thead>
<tr>
<th>Compound</th>
<th>HepatoCells TC50 (M)</th>
<th>Primary Hepatocytes TC50 (M)</th>
<th>HepG2 Cells TC50 (M)</th>
<th>Literature TC50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>9.37E-3</td>
<td>6.79E-4</td>
<td>4.42E-4</td>
<td>&gt;1.0E-4</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>-9.82E-3</td>
<td>-1.09E-4</td>
<td>-4.19E-5</td>
<td>&gt;1.0E-5</td>
</tr>
<tr>
<td>Tacrine</td>
<td>-6.76E-4</td>
<td>9.68E-4</td>
<td>6.40E-4</td>
<td>&gt;1.0E-4</td>
</tr>
<tr>
<td>Menthol</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

TC50 values were generated from the area under the curve integration of the kinetic DMR responses observed with primary hepatocytes, Corning HepatoCells and the kinetic HepG2 cells using Epic technology. These values were in agreement with TC50 values found in the literature from the 24-hour toxicity studies.

**Conclusions**

- Toxicity assays can be performed using the Corning Epic technology and the Corning Epic BT Reader, which is amenable to running assays at 37°C.
- Corning Epic microplates can be coated using a variety of surfaces to support the growth and label-free assays of various primary and immortalized cell types, including hepatic models.
- Corning Epic technology supports hepatotoxicity assays utilizing primary hepatocytes, immortalized Corning HepatoCells, and hepatocellular carcinoma cell lines, such as HepG2.

**References**


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