CHCP-I-T  Cryopreserved Plateable Cynomolgus Hepatocytes for Induction and Transporter assays
Cell Specification

Species: Macaca fascicularis  Serology: negative for Filovirus/Ebola-like, SRV, SIV, STLV-1
Gender: male  Age: 6 years 5 months

Cryopreservation:
Date: July 17, 2012
Amount per vial: 10 x 10^6 cells

Thawing: n=4
Post-thaw viability: 88.9 ± 3.9 %
Post-thaw yield per vial: 5.6 ± 0.4 x 10^6 cells
Recovery: 56 %

Phase contrast on day 1 after thawing

Phase contrast on day 2 after thawing

Recommended seeding density on collagen-coated plates: 290,000 cells per cm^2
Culture in Human Hepatocyte Maintenance Medium (HHMM).

CYP P450 activity in culture after thawing:
Ethoxyresorufin-O-deethylation:
Induction with 10 µM beta-naphthoflavone
Induction with 25 µM beta-naphthoflavone

<table>
<thead>
<tr>
<th>Activity</th>
<th>pmol/(mg × min)</th>
<th>x-fold induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2 ± 0.9</td>
<td>13.6</td>
<td></td>
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<tr>
<td>7.3 ± 0.3</td>
<td>18.9</td>
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</tbody>
</table>

Uptake transporters: uptake of Estrone 3-sulfate (E3S) in cryopreserved hepatocytes after 2 min incubation.

<table>
<thead>
<tr>
<th>Activity</th>
<th>pmol/(mg × min)</th>
<th>x-fold induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>13.9 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Cryopreserved</td>
<td>5.9 ± 1.4</td>
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</tbody>
</table>

Note: Yield, viability, recovery and activity assays were performed at PRIMACYT using PRIMACYT’s manual for thawing, plating and culture of primary cryopreserved hepatocytes.

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