Cryopreserved Human Hepatocyte High-Throughput Screening Protocol: 96-Well ATP Cytotoxicity Assay

Introduction
Cryopreserved human hepatocytes represent a relevant experimental model for the evaluation of acute hepatotoxic potential of test articles in man.1,2

Purpose
This assay is designed to screen for the cytotoxic potential of test articles in human hepatocytes.

Principle of the Procedure
Cellular adenosine triphosphate (ATP) content is a marker of cellular energy status and viability. When cells undergo necrosis or apoptosis, their ATP levels decline rapidly. In combination with luciferase, the addition of D-luciferin to cells generates light in the presence of ATP. The intensity of the luminescence is proportional to the intracellular ATP content.3,4

Materials*

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Name/Catalog/Model #</th>
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</thead>
<tbody>
<tr>
<td>ATPLite</td>
<td>Perkin Elmer Life Sciences</td>
<td>6016941</td>
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<tr>
<td>Cryopreserved human hepatocytes</td>
<td>BioreclamationIVT</td>
<td>M00995, F00995</td>
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<tr>
<td>Orbital shaker</td>
<td>Bellco Glass Inc.</td>
<td>7744-01000</td>
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<td>96-well opaque plastic plates</td>
<td>Costar</td>
<td>3915</td>
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<tr>
<td>Chlorpromazine</td>
<td>Sigma Chemical Co.</td>
<td>C-8138</td>
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<tr>
<td>Wallac Victor® Multilabel Counter</td>
<td>Wallac</td>
<td>1420-040</td>
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Solvents/Solubilizers
- Methanol: Fisher Scientific A-452-4

Buffers
- InVitroGRO™ HI Medium: BioreclamationIVT Z90009
- Krebs-Henseleit Buffer powder (KHB): Sigma Chemical Co. K-3753
- Amikacin sulfate: Sigma Chemical Co. A-2324
- Calcium chloride dihydrate: Sigma Chemical Co. C-3881
- Gentamicin sulfate: Sigma Chemical Co. G-3632
- N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonate) (HEPES): Sigma Chemical Co. H-3375
- Heptanoic acid: Sigma Chemical Co. H-9378
- Hydrochloric acid (HCl): JT Baker 9535-01
- Sodium bicarbonate: Sigma Chemical Co. S-5761
- Sodium hydroxide (NaOH): Sigma Chemical Co. S-9625

*Items listed in this Materials section are for convenience; suitable materials and equipment from other manufacturers may be substituted as appropriate. Contact information for vendors used by BioreclamationIVT are listed in the Notes section at the end of this document.
Procedure

Reagent Preparation

(To be completed in advance of assay.)

1. Prepare KHB by supplementing it with
   • amikacin sulfate (84 µg/mL)
   • calcium chloride (1 mM)
   • HEPES (20 mM)
   • gentamicin sulfate (84 µg/mL)
   • heptanoic acid (4.2 µM)
   • sodium bicarbonate (2.2 g/L).

   Keep KHB at a pH of 7.3. Use 1N NaOH to raise pH. Use 1N HCl to lower pH.

2. Prepare stock solutions of test articles and positive and negative controls. Stock solutions should be made at 100X, using an appropriate organic solvent (e.g., DMSO). Chlorpromazine is recommended as the positive control (final concentration = 100 µM). For a negative control, use KHB alone for water-soluble test articles or KHB with 1% solvent for lipophilic test articles.

Assay

3. Thaw cryopreserved human hepatocytes according to instructions provided by BioreclamationIVT (see Storing and Thawing Cryopreserved Hepatocytes).

4. Re-suspend hepatocytes in KHB at a density of 1.0 × 10⁶ cells/mL.

5. Add 50 µL of 2X test article solution per well (dissolved in KHB with 1% solvent; final cell density = 50,000 cells/100 µL; final test article concentration = 1X) to a 96-well opaque plate pre-warmed to 37 °C.

6. Load 50 µL of hepatocytes per well.

7. Place the plate in an incubator at 37 °C, 5% CO₂ for 2-4 hours.

8. Add 50 µL of Mammalian Cell Lysis Solution per well. Shake for 2 minutes at 700 rpm on a room temperature orbital shaker.

9. Add 50 µL of reconstituted substrate solution (see kit instructions) per well. Shake at 700 rpm for 2 minutes. Keep the plate in the dark for 10 minutes.

10. Measure the luminescence in each well.

11. Compare luminescence of the treated samples to the untreated controls to determine the percent viability.
Note: The ATPLite kit contains standards that can also be used to determine μM ATP in each well based on a standard curve.

References

