Protocol for use No. 4: upcyte® Hepatocyte Cytotoxicity Testing

Introduction

This PFU describes how to perform a cytotoxicity assay using upcyte® Hepatocytes cultured in 96-well plates.

Required products

- **upcyte Hepatocytes (cryopreserved)**

Each vial contains ~5x10^6 upcyte Hepatocytes (upcyte; "upregulated hepatocytes") frozen per vial from which at least 50% recovery is expected after thawing.

- **Hepatocyte Thawing Medium**

This is ready-to-use for thawing upcyte Hepatocytes. No additional supplements are required.

- **Hepatocyte High Performance Medium**

The Hepatocyte High Performance Medium is designed for the optimal culture and incubation of compounds with upcyte Hepatocytes. In order to obtain Hepatocyte High Performance Medium add the entire contents of supplement A and L-Glutamine to the basal medium.

Due to the manufacturing process the medium may appear opaque but this does not affect the performance of the cells.

The shelf life of the fully supplemented medium is 6 weeks. Once fully supplemented do not freeze the Hepatocyte High Performance Medium. Add antibiotics if preferred.

- **Collagen coated (Type I) culture plates**

These are not provided by BioIVT. Either use pre-coated plates or prepare them as follows: dilute collagen type I with 0.02M acetic acid to a final concentration of 50µg/mL. Add 0.1mL/cm^2 of the diluted collagen solution to the culture dishes and incubate for 1h at room temperature. Wash the plate twice with PBS and use directly or air dry before storing at 4°C.

Storage conditions

upcyte Hepatocytes can be stored in liquid or vapour phase nitrogen. They should not be stored at -70°C.

Store basal medium as well as fully supplemented Hepatocyte High Performance Medium protected from light at 2 − 8°C. Store Supplement A at -20°C. The expiration date is indicated on the label of the basal medium as well as on the supplement label.

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Thawing of cryopreserved upcyte Hepatocytes

1. Thaw and seed upcyte hepatocytes according to **PFU No. 12**.
2. Incubate cells for 7 days with medium change according to **PFU No. 12**.

Treatment of upcyte Hepatocytes for cytotoxicity screening

1. Remove the medium and replace with 0.15mL Hepatocyte High Performance Medium containing either vehicle control or test compound.
2. We recommend incubating treated cells for 72-96h.
3. Determine the viability of cells using standard endpoint measurements (e.g. ATP, LDH, MTT/MTS).

Results using upcyte Hepatocytes

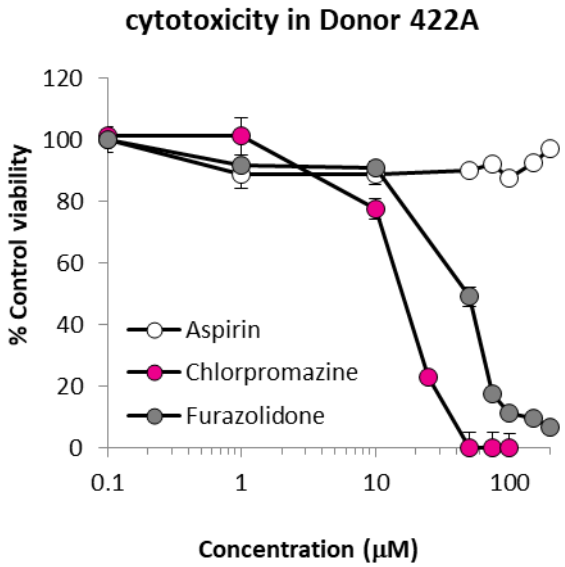
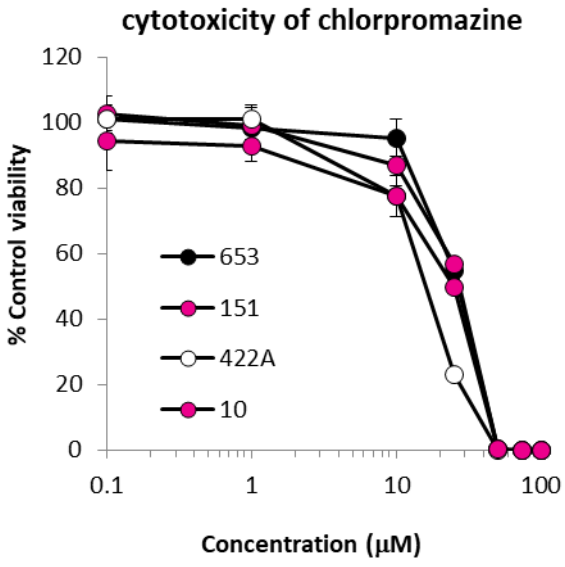


Figure 1 shows an example of the cytotoxicity of chlorpromazine, furazolidone and aspirin in upcyte Hepatocytes from Donor 422A. This shows that these cells can discriminate between toxic and non-toxic compounds.



The cytotoxicity of chlorpromazine in different upcyte Hepatocyte donors is shown in Figure 2. The EC_{50} for this compound using ATP as the endpoint is between 16 – 30 □M.

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Product information

Product	Supplements/Components	Product number
upcyte Hepatocytes	• cryopreserved (1mL)	CHE002
Hepatocyte Thawing Medium	• ready-to-use (50mL)	MHE001
Hepatocyte High Performance Medium	• Basal Medium (500mL) • Supplement A (5mL) • L-Glutamine (5 mL)	MHE003

Purchaser Notification

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(b) for resale; or
(c) for the production of therapeutic, diagnostic, prophylactic or any other products; or
(d) to provide a service to deliver information or materials to a third party.
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