Predictive 3D Hepatotoxicity Assay Service
using HepG2 Cells in Alvetex 96-well plate format

REPROCELL discovery

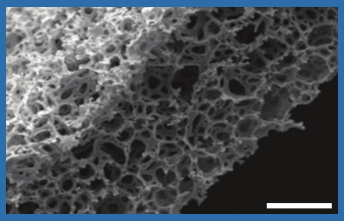
REPROCELL's new state-of-the-art **Centre for Predictive Drug Discovery** now offers 3D assay services to meet the contract research needs of academic and industrial customers. Our scientists have developed a 96-well plate format 3D hepatotoxicity assay, utilising HepG2 cells grown in our own Alvetex™ 3D cell culture scaffold.

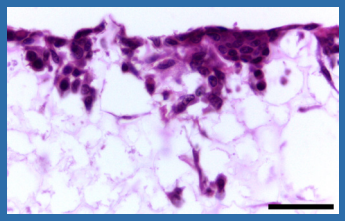
Our team of expert scientists are available to discuss how this assay can answer your contract research needs and to design a protocol that will meet your study requirements.

Key Features and Benefits

- Increase confidence and reduce drug development costs with a good *in vitro* prediction of clinical drug induced liver injury (DILI).
- Save money and time with a large number of test wells and a small compound volume.
- Assess chronic drug toxicity with cells stable for at least 28 days, allowing multiple days dosing.
- Innovation award-winning 3D substrate supported by multiple peer-reviewed publications.

Genuine 3-dimensional growth substrate in 96-well plate format





Left: Alvetex Scaffold 96-well plate.

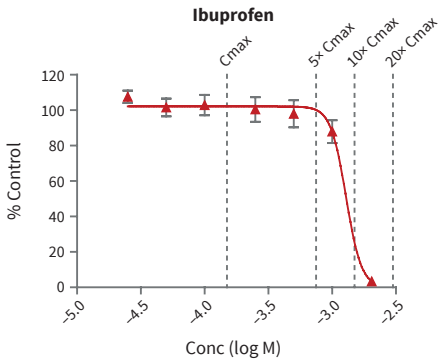
Middle: Scanning electron micrograph shows the highly porous architecture of Alvetex. Scalebar = 100 microns.

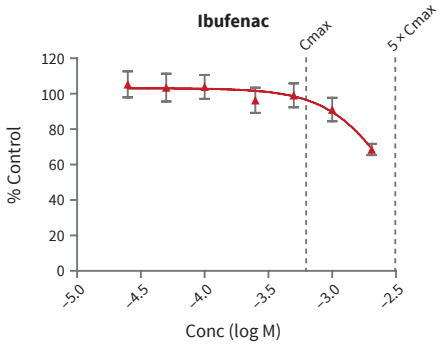
Right: Histological staining of HepG2 cells grown on Alvetex Scaffold 96-well plate for 7 days. Scalebar = 50 microns.

DILI compound specificity and sensitivity

Compound	EC_{90} 3D Alvetex (µM)	5× Clinical Cmax (µM)*	Clinical +ve or –ve DILI	Correctly Predicted
Ibuprofen	948.4	749.0	Negative	Yes
Ibufenac	837.5	3125.0	Positive	Yes
Entacapone	51.3	30.0	Negative	Yes
Tolcapone	24.6	109.5	Positive	Yes
Rosiglitazone	>75	5.2	Negative	Yes
Troglitazone	72.4	32.0	Positive	No
Acetaminophen	8871.6	650.0	Positive	No
Gemfibrozyl	496.6	700.0	Positive	Yes

Above: Table of EC_{90} (10% cell death) values for HepG2 cells pre-cultured in Alvetex Scaffold 96-well plates for 7 days and subsequently exposed to DILI +ve or –ve compounds for a further 7 days. Note that 3/3 DILI –ve and 3/5 DILI +ve compounds are correctly predicted (cell viability assessed by Promega CellTiter-96 Aqueous Cell Proliferation Assay, single EC_{90} value calculated from an average of n = 4 replicate wells +/- standard error, * clinical Cmax values from Bale *et al.*, 2014, *Exp. Biol. Med.* 239(9):1180-1191). HepG2 offers an inexpensive and robust high screening system widely used in industry. It is however recognised that it has some limitations (Gerets *et al.*, 2012, *Cell Biol. Toxicol.* 28:69-87)





Right: Example concentration-response curves. Ibuprofen and Ibufenac shown.

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REPROCELL Discovery provides cutting-edge stem cell products and services, human biospecimens, and contract research for pre-clinical drug discovery

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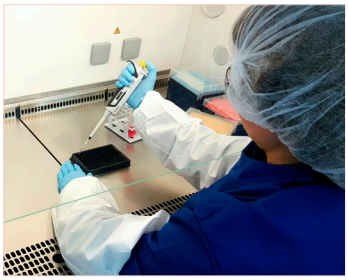
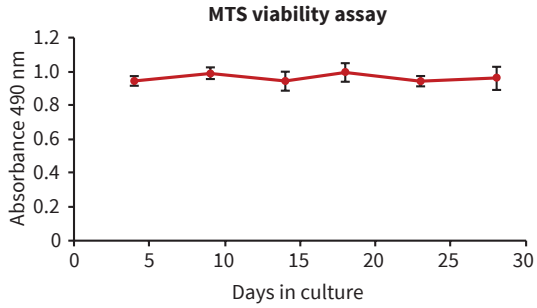
State-of-the-art facilities



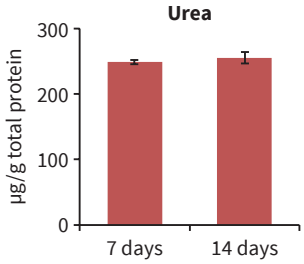
Above: All our service assays are conducted at REPROCELL's Centre for Predictive Drug Discovery, Glasgow, UK.

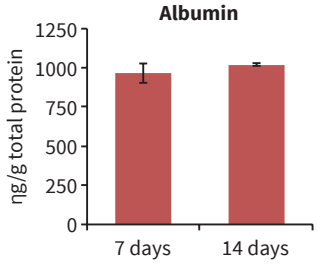
Long-term cell viability enables long-term repeat drug dosing

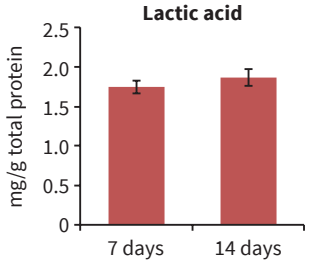


Above: HepG2 cells grown in Alvetex Scaffold 96-well plates show sustained viability for at least 4 weeks, thus enabling cytotoxicity or functional testing over long-time periods (cell viability assessed by Promega CellTiter-96 Aqueous Cell Proliferation Assay, average of n=3 +/– standard error).

Stable expression of liver function and cell health markers



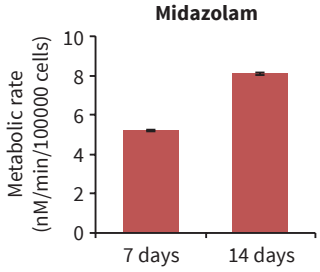


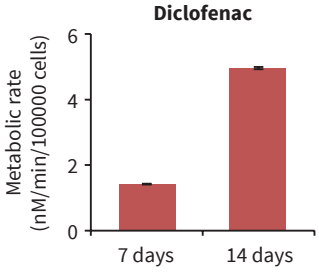


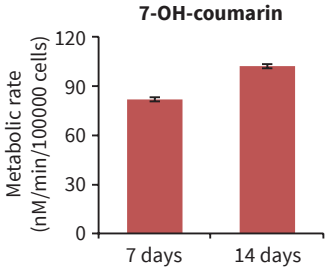
Left: HepG2 cells grown in Alvetex Scaffold 96-well plates produce urea, lactic acid and albumin for up to at least 14 days, with no decrease in secretion between 7 and 14 days of culture (levels assessed by Universal Biologicals Quantichrom Urea Assay Kit, Megazyme Lactic Acid Assay Kit and Universal Biologicals Human Albumin ELISA Assay, normalised to total protein levels, average of n=3 +/- standard error).

Sustained CYP and Phase II enzyme metabolism

Right: HepG2 cells metabolise midazolam (CYP3A4 substrate), diclofenac (CYP2C9 substrate) and 7-OH-coumarin (phase II enzymes substrate) when pre-cultured in Alvetex Scaffold 96-well plates for up to at least 14 days. Note the increased metabolism of substrates between 3 and 14 days (substrate degradation assessed by LC-MS-MS after 1h exposure to either 12.5 µM midazolam, 25 µM diclofenac or 75 µM 7-OH coumarin average of n = 3 +/- standard error).







Take advantage of our innovation award-winning 3D substrate to explore our assay development capabilities in other tissue types, according to your needs and as already exemplified by multiple peer-reviewed publications.