

## Introduction

This TA gives advice how to perform a co-culture using upcyte® Hepatocytes and upcyte® LSECs cultured in various formats. Before seeding the cells for the experiment, an initial sub-culture is performed to increase recovery after cryopreservation. Every 3D and/or co-culture system is complex, has its own requirements and is treated differently. Treatment & handling of the cells is dependent on the system used as well as the endpoint of your experiment. There is no standard guideline. Here we summarized some general advice. Please contact us if you have any questions ([info@upcyte.com](mailto:info@upcyte.com) phone.: +49 40 334647370)

## Required products

### HEPATOCYTES

- upcyte® Hepatocytes (cryopreserved #CHE001)
- Hepatocyte High Performance Medium (500mL #MHE003)
- PFU No. 12 for upcyte® Hepatocytes

### LSECs

- upcyte® LSECs (cryopreserved #CLS002)
- LSEC Culture Medium (100mL #MLS002)
- PFU No. 30 for upcyte® LSECs

## BOTH CELL TYPES

### Hepatocyte & LSECs Co-Culture Medium (500ml #MHE004)

This Medium is designed for the optimal culture and endpoint measurement of upcyte® Hepatocytes & LSECs. This medium consists of ½ Hepatocyte High Performance Medium and ½ LSECs Culture Medium. Either you mix those two media or you obtain the Co-Culture Medium separate. In order to obtain the complete Medium, centrifuge Suppl. B & C (25µl, droplets might stick to the wall of the vial) and add the entire contents of supplement A, B, C, FBS and L-glutamine to the basal medium.

### Hepatocyte & LSECs Thawing Medium (50ml #MHE001)

A ready-to-use formulation for thawing upcyte® Hepatocytes & LSECs. No additional supplements are required.

**Storage of cryopreserved cells:** upcyte® Hepatocytes & LSECs should be stored in liquid or vapour phase nitrogen.

**Storage of media:** Store Basal Medium, Thawing Medium and fully supplemented Cp-Culture Medium protected from light at 2–8°C. Store Supplements at -20°C. The expiration date is indicated on the respective label.

**Shelf Life of media:** The shelf life of the fully supplemented media is 6 weeks. Do not freeze upcyte® Media. Add antibiotics only if it is necessary for your experiments.

## Additional products not supplied by upcyte technologies GmbH:

- Foetal bovine serum (FBS) for quenching trypsin activity after detachment
- PBS without calcium/magnesium
- Trypsin/EDTA (0.05% /0.02% EDTA)
- Collagen coated (type I) culture vessels for both cell types

*Coated culture vessels can either be bought (e.g. from Corning, Bedford, MA, USA) or self-prepared. For coating, dilute collagen type I (e.g. Sigma-Aldrich, C3867) with 20 mM acetic acid to a final concentration of 50 µg/ml. Add 0.1 ml/cm<sup>2</sup> of the diluted collagen solution to the culture dishes and incubate for 1 h at RT. Wash the plate twice with PBS and use directly or air dry before storing at 4°C.*

### Day 1

Thaw and seed upcyte® hepatocytes according to [PFU No. 12](#) in cell culture flasks. Thaw and seed upcyte® LSECs according to [PFU No. 30](#) in cell culture flasks.

### Day 2

The next day perform a medium change for both cell types according to the respective PFU. The additional culture time is needed to get rid of apoptotic cells as well as giving the cells enough recovery time after cryopreservation. We have seen that especially for the hepatocytes this is a crucial step.

### Day 4

Detach the cells using trypsin according to the respective protocols.

**Different handling:** Please note the differences in Trypsin/EDTA incubation times (3-4min for hepatocytes, 3-5min for LSECs) and especially the difference in centrifugation speed (90 x g for hepatocytes, 280 x g for LSECs). Resuspend both cell types in Co-Culture Medium after the centrifugation step.

**Cell ratio:** Since the liver consist of around 15% of LSECs and 80% of hepatocytes, we recommend a ratio of around 1:5 (20.000 LSECs : 100.000 Hepatocytes). We have not yet tested the addition of other cell types such as Kupffer Cells and Stellate Cells.

**Seeding order:** If you are performing a [2D monolayer culture](#), we recommend passaging the upcyte® LSECs first and seeding them into your final well format first. Give them an adherence time of around 2h before seeding the hepatocytes gently on top. If you are using e.g. spheroid cultures, passage both cell types at the same time and mix before seeding.

**Start of the experiment:** Wait at least another 48h until you start your experiment. (**Day 6**)

### Tested co-culture formats

<b>Organoid cultures</b> (Matrigel coated 24 well plates)	Please refer to our Application Note 7 & 2015 Ramachandran <i>et al.</i> , (PLOS ONE   DOI:10.1371/journal.pone.0139345)
<b>Fluidic systems</b> (e.g. Dynamic42 Chip System)	Please refer to literature, e.g. Gröger & Rennert <i>et al.</i> , Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model. (Sci Rep. 2016 Feb 23;6:21868.)

### Other possible formats

<b>Spheroid cultures</b> (low attachment plates)	Please refer to the literature. We have tested mono-cultures of hepatocytes in low attachment plates and spheroids form nicely. Ask for poster No. 14.
<b>3D printing</b>	Please refer to the literature. Since our cells are quite robust, we are confident they perform well in printed tissue. We are currently in a collaboration to generate data.
<b>Scaffolds</b> (e.g. Matrigrids)	Manuscript is currently in preparation. Please note that the scaffolds/matrices need to be collagen coated for the cells to adhere.

Product information

Product	Supplements/Components	Product number
upcyte® Hepatocytes (cryopreserved)	<ul style="list-style-type: none"> <li>• Cryopreserved vial, 5 Mill cells (1ml)</li> </ul>	CHE002
Hepatocyte & LSECs Thawing Medium	<ul style="list-style-type: none"> <li>• ready-to-use (50mL)</li> </ul>	MHE001
Hepatocyte High Performance Medium	<ul style="list-style-type: none"> <li>• basal Medium (500mL, opaque)</li> <li>• Supplement A (5mL)</li> <li>• L-Glutamine (5mL)</li> </ul>	MHE003
upcyte® LSECs (cryopreserved)	<ul style="list-style-type: none"> <li>• Cryopreserved vial, 2 Mill cells (1ml)</li> </ul>	CLS002
LSECs Culture Medium	<ul style="list-style-type: none"> <li>• 1 bottle basal medium (100mL, red)</li> <li>• 1 Supplement A (10µL)</li> <li>• 1 Supplement B (10µL)</li> <li>• 1 FBS (10mL)</li> </ul>	MLS002
Co-Culture Medium	<ul style="list-style-type: none"> <li>• 1 bottle basal medium (500mL, light red)</li> <li>• Supplement A (2,5mL)</li> <li>• Supplement B (25µL)</li> <li>• Supplement C (25µL)</li> <li>• FBS (25mL)</li> <li>• L-Glu (2,5mL)</li> </ul>	MHE004

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