# Protocol for use No. 30:
# upcyte® LSECs

upcyte technologies

(v1.1 – 03.12.2015)

## upcyte® LSECs

upcyte® Liver Sinusoidal Endothelial Cells (upcyte® LSECs) can be stored in liquid or vapour phase nitrogen. They should not be stored at -70°C.

## Liver Sinusoidal Endothelial Cell Culture Medium

The LSECs Culture Medium is designed for the optimal culture and expansion of upcyte® LSECs.
LSECs Culture Medium consists of LSEC Basal Medium plus Supplement A&B and FBS. In order to obtain complete LSEC Culture Medium, ALL supplements need to be added to the basal medium entirely.

**Storage**: Store the basal medium protected from light at 2 – 8 °C. Store FBS and the supplements A and B immediately after arrival at -20 °C. Do not freeze the basal medium. If stored properly, the products are stable until the expiry date stated on the label. After adding FBS, and the supplements A and B to the basal medium, the shelf life of the LSECs Culture Medium protected from light is 6 weeks at 2 – 8 °C.
*FBS and supplement A and B can be thawed on arrival. They can be re-frozen without losing any activity.*

## Thawing of cryopreserved upcyte® LSECs

**Note: Use collagen-coated (Type I) culture plates.**

1. Pre-warm LSEC Culture Medium to 37°C.
2. Carefully remove the cryovial from the storage tank. This should only take seconds.
3. Thaw cells in a 37°C water bath and continuously agitate slowly for 90 - 120 sec. A small piece of ice should be visible.
4. Spray 70% ethanol on the vial to avoid microbial contamination. Transfer the vial to a laminar flow-hood.
5. Transfer the thawed cell suspension (1mL) from the cryovial into 10mL LSEC Culture Medium in a 50mL tube by gently pipetting the cells into the medium with a 2mL pipette.
6. Using a 1mL pipette, transfer 1mL of the cell suspension back to the cryovial and pipette the content back into the tube. Pellet the cells by centrifuging at 620 x *g* for 5 min at RT.
7. Aspirate the supernatant without disrupting the pellet. Leave approximately 200µL medium on top of the cells.
8. Add 800µL of pre-warmed LSEC Culture Medium to the pellet and resuspend the cells by pipetting them gently up and down for 1 - 2 times.
9. Determine cell number by e.g. using a Neubauer haemocytometer.

## Plating of upcyte® LSECs

The cells should be seeded at a density of 5,000 cells/cm$^2$. Cells are cultured as adherent monolayer on collagen-coated plates and have a doubling time of ~3days.

1. Dilute upcyte® LSECs to 5,000 cells/cm$^2$ in pre-warmed LSEC Culture Medium. Culture the upcyte® LSECs for 24h in a humidified incubator under an atmosphere of 95% air and 5% $CO_2$.
2. Change medium the day after thawing.
3. Replace medium every other day with fresh LSEC medium afterwards.
4. Perform your experiments.

## Sub-culture of upcyte® LSECs

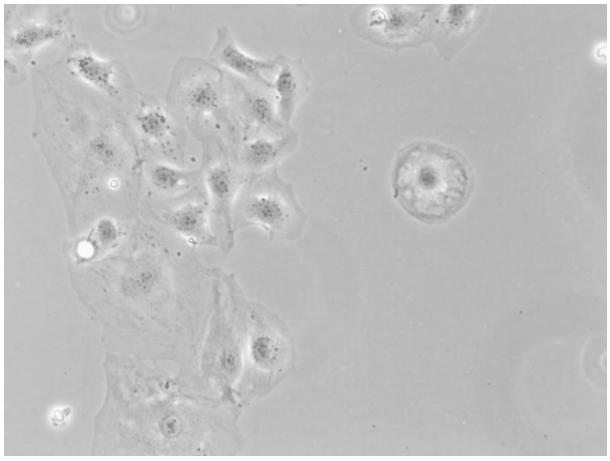
To obtain more cells, upcyte® LSECs can be passaged one time at 80 - 90% confluence.

1. Pre-warm LSEC Culture Medium to 37°C.
2. Aspirate medium from the plate.
3. Wash the plate once with PBS.
4. Aspirate PBS and add an appropriate volume of 1x trypsin / EDTA solution per culture dish (~20µL/cm$^2$).
5. Incubate 3 - 5 min at 37°C until most of the cells are rounded up (check under the microscope).
6. Gently tap the cell culture vessel to detach rounded up cells .

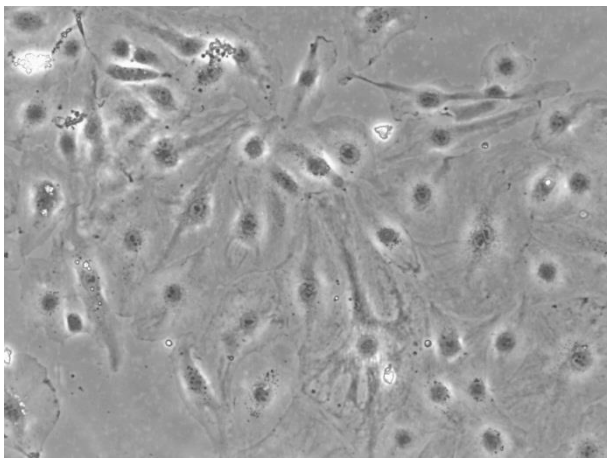
7. Add pre-warmed LSEC Culture Medium at an equal volume of the trypsin solution used in step 4 and rinse the remaining attached cells from the culture surface.
8. Transfer the complete suspension to a tube and centrifuge for 5 min 620 x *g* at RT.
9. Discard supernatant and add pre-warmed LSEC Culture Medium.
10. Carefully resuspend the pellet and determine the cell number as described in the thawing section (step 7 - 9).
11. Seed approx. 5,000 cells/cm$^2$ in LSEC medium.
    Note: upcyte® LSECs are able to perform up to 3 population doublings (1 Passage). Afterwards cells become senescent and lose their biological functionality.

**Morphology of upcyte® LSECs**

Cells spread over the plate when they have space but become smaller and might compact their cytoplasm when getting confluent.



One day after seeding (5,000 cells/cm$^2$)



At confluency (~15,000 cells/cm$^2$)

*Unless indicated otherwise, upcyte technologies products and services are for research purpose only. Do not use for diagnostic or therapeutic applications.*

## Product information

| Product | Supplements/Components | Product number |
|---|---|---|
| **upcyte® LSECs cryopreserved** | • 5 Million cells | CLS001 |
| **Liver Sinusoidal Endothelial Cell (LSEC) Culture Medium 100mL** | • LSEC Culture Medium (100mL) basal<br>• Supplement A&B, FBS | MLS002 |
| **Liver Sinusoidal Endothelial Cell (LSEC) Culture Medium 500mL** | • LSEC Culture Medium (500mL) basal<br>• Supplement A&B, FBS | MLS003 |

## Purchaser Notification

**Limited Use Label License (upcyte technologies)**

This cellular product (upcyte® cells) generated using the upcyte® technology is provided under an intellectual property license from upcyte technologies GmbH. The transfer of the upcyte® cells is conditioned on the buyer using the purchased product solely in research conducted by the buyer, and the buyer must not use, sell or otherwise transfer this product, its components or any data generated with the product
(a) for diagnostic, therapeutic or prophylactic purposes; or
(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.
This intellectual property license is timely limited and granted to the buyer for 12 month after delivery of the upcyte® cells. After this time the buyer has to destroy remaining upcyte® cells and populations thereof or obtain a follow-up license. For information on purchasing a license to this upcyte technologies product for other purposes, for an extended time frame and/or for commercial purposes, contact upcyte technologies GmbH, Osterfeldstraße 12-14, 22529 Hamburg, Germany or info@upcyte.com.

**Limited Use Label License (Life Technologies Corporation)**

During the generation of these upcyte® cells a product licensed from Life Technologies Corporation containing the following Limited Use Label License was used:

This product is provided under an intellectual property license from Life Technologies Corporation. The transfer of this product is conditioned on the buyer using the purchased product solely in research conducted by the buyer, and the buyer must not use, sell or otherwise transfer this product or its components for (a) diagnostic, therapeutic or prophylactic purposes; or (b) resale, whether or not resold for use in research or (c) or for the commercial production of therapeutic, diagnostic, or prophylactic products or other products not intended for research use; or (d) to provide a service to deliver information or materials to a customer other than for research purposes of such customer. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@lifetech.com.