Protocol for use No. 30:
upcyte® LSECs

upcyte technologies

(v1.8 – April 26th 2021)

When breaking the sealed bag of upcyte® cells you are explicitly accepting the terms of the limited use label license provided with the purchase of the cells. IT IS STRICTLY PROHIBITED TO EXPAND THE CELLS.

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

Introduction

This PFU describes how to culture upcyte® Liver Sinusoidal Endothelial Cells (LSECs) as 2D monolayers for endpoint measurements. Before seeding the cells for the experiment, an initial sub-culture is performed to increase recovery after cryopreservation.

Required products for upcyte® LSEC culture

upcyte® LSECs ($2 \cdot 10^6$ frozen cells)
Each vial contains $2 \cdot 10^6$ frozen upcyte® LSECs from which at least 70% recovery is expected after thawing. The protocol includes one sub-culture step. **THE CELLS ARE NOT FOR FURTHER EXPANSION.**
Storage: upcyte® LSECs should be stored in liquid or vapour phase nitrogen.
Shelf life: 2 years after receipt.

Hepatocyte/LSECs Thawing Medium (50ml)
Our Hepatocyte/LSECs Thawing Medium is a ready-to-use formulation for thawing LSECs, no additional supplements are required.
Storage: Store Thawing Medium protected from light at 2−8°C.
Shelf life: The expiration date is indicated on the respective label.

LSEC Culture Medium (100ml or 500ml)
Our LSEC Culture Medium is designed for the optimal culture and expansion of upcyte® LSECs. The medium consists of LSEC Basal Medium plus Supplements A/B and FBS. In order to obtain the complete Culture Medium, centrifuge the supplements A/B (collect all droplets on the bottom) and add A/B & FBS to the basal medium.
Storage: Store the basal medium protected from light at 2−8°C and the supplements/FBS immediately after arrival at -20°C. The expiration date is indicated on the respective label.
Shelf life: The shelf life of the fully supplemented media is 6 weeks. Do not freeze the media.

Note: Our Media do not contain antibiotics. Add antibiotics only if it is necessary for your experiments.

Additional products not supplied:

- PBS without Ca^{2+} or Mg^{2+}
- Trypsin-EDTA (0.05% Trypsin / 0.02% EDTA)
- Collagen coated (type I) culture vessels

Coated culture vessels may be purchased from vendors like Corning or prepared by diluting 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^2 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.

Culture protocol

Day 1: Thawing of cryopreserved upcyte® LSECs (e.g. Monday)

1. Pre-warm Thawing Medium to RT and LSEC Culture Medium to 37°C.
2. Carefully remove the cryovial from the storage tank. This should only take seconds; longer times will decrease the cell yield.
3. Thaw cells in a 37°C water bath for up to 2 min. A small piece of ice should be visible.
4. Thoroughly disinfect the vial using 70% ethanol, wipe and transfer to a laminar flow-hood.
5. Transfer the thawed cell suspension (1 mL) from the cryovial into 20 mL Thawing Medium in a 50 mL tube by gently pipetting the cells into the medium with a 2 mL pipette.
6. Using a 1 mL pipette, transfer 1 mL of the cell suspension back to the cryovial and pipette the content back into the tube. Pellet the cells by centrifuging at **280 x *g* for 5 min** at RT.
7. Aspirate the supernatant without disrupting the pellet. Leave approximately 200 µL medium on top of the cells.
8. Add pre-warmed LSEC Culture Medium for a final volume of ~1 ml per 1 million cells to the pellet and resuspend the cells by pipetting them gently up and down for 2-3 times.
9. Determine cell number by e.g. using a Neubauer haemocytometer.
10. Dilute upcyte® LSECs to 10,000 cells/cm^2 in pre-warmed LSEC Culture Medium in **collagen coated** cell culture flasks (e.g. T175) or appropriate cell culture dishes.
11. Culture the upcyte® LSECs for 24 h in a humidified incubator under an atmosphere of 95% air and 5% CO_2.

Note: We recommend a pre-culture of 4-5 days to ensure sufficient recovery of the cells. upcyte® LSECs show a doubling time of approximately 2-3 days and are able to perform up to 2 population doublings until their functionality is reduced and cells will start to become senescent.

Day 2: Medium change (e.g. Tuesday)

12. Change medium the next day.
13. Culture the cells for additional 2-3 days until reaching 70-80% confluence. Cells are subsequently passaged using trypsin. Do not expand the cells further. Replace medium every other day with fresh medium afterwards.

Note: PD time is strongly donor dependant and growth might be reduced after thawing. If the cells did not double enough, wait another day.

Day 4/5: Seeding into multiwell plates or format of your choice (e.g. Friday)

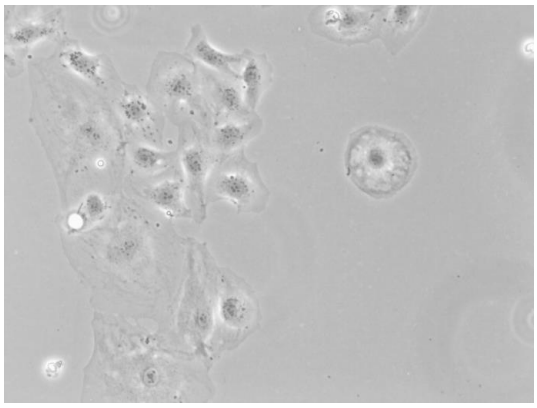
14. Pre-warm LSEC Culture Medium, PBS, trypsin/EDTA to 37°C.
15. Carefully aspirate the culture supernatant.
16. Wash the plate once with 100 µl PBS/cm^2.
17. Add 20 µl/cm^2 trypsin/EDTA
18. Incubate for 3-5 min at 37°C until most of the cells are rounded up and detached. Avoid incubating the cells for more than 7 min.
19. Gently tap the cell culture vessel to detach rounded up cells.
20. Stop the trypsin activity by adding twice the volume of LSECs Culture Medium.
21. Rinse the remaining attached cells from the culture surface.
22. Transfer the complete suspension to a tube and centrifuge for **5 min 280 x *g*** at RT.
23. Discard supernatant and add pre-warmed LSEC Culture Medium.
24. Carefully resuspend the pellet and determine the cell number as described in the thawing section (steps 7-9).
25. Seed approx. 10,000-30,000 cells/cm^2 in medium in your final format. The amount of cells used is depending on your fomat as well as endpoint.

Day 8: Start performing your assay of choice (e.g. Monday)

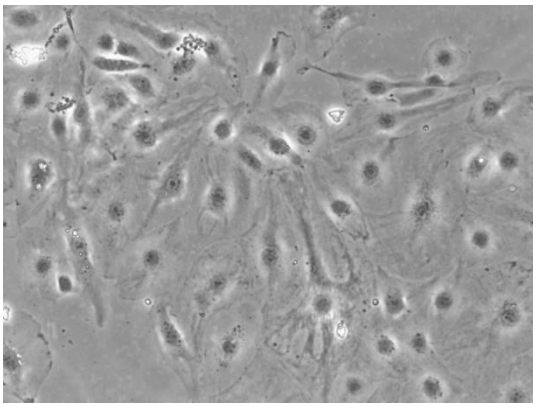
upcyte® LSECs may be used for various applications, including immunological responses and cytotoxicity testing. **Note:** Our LSECs are suitable for co-culture studies with upcyte® hepatocytes. Please ask for our Technical Advice TA01.

Expected morphology of upcyte® LSECs

upcyte® LSECs spread over the plate when they have space but start to “compact” their cytoplasm when getting confluent.



One day after seeding (10,000 cells/cm^2).



At 70-80% confluency (~30,000 cells/cm^2).

Product information

Product	Supplements/Components	Product number
upcyte® LSECs cryopreserved	• 2 Million cells	CLS002
Hepatocyte/LSECs Thawing Medium 50mL	• 1 tube ready-to-use medium (50 mL, opaque)	MHE001
LSEC Culture Medium 100mL	• 1 bottle basal medium (100 mL) • Supplement A&B (10 µL each) • FBS (10 mL)	MLS002 (100 mL)
LSEC Culture Medium 500mL	• 1 bottle basal medium (500 mL) • Supplement A & B (50 µL each) • FBS (50 mL)	MLS003 (500 mL)

Purchaser Notification

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(b) for resale; or
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(d) to provide a service to deliver information or materials to a third party.
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