Cell Encapsulation to Generate Patient-Derived Organoids (PDOs) in LunaX™ ECM

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Introduction

This protocol describes the encapsulation of primary tumour-derived cells in LunaX CoreMatrix Low Stiffness ECM to establish patient-derived organoids (PDOs) in 3D culture. The workflow is designed to support the formation and maintenance of tumour-relevant 3D structures within a defined, tuneable ECM hydrogel environment.

This protocol is suitable for oncology research, drug discovery, and preclinical model development using LunaX CoreMatrix Low Stiffness kits and the LunaX Crosslinker. It is intended for use with primary tumour-derived cells isolated and expanded according to LX-APR-001. Culture conditions, seeding density, matrix stiffness, and assay duration should be optimised according to tumour type, cell availability, and the intended downstream application.

Expected Results

Patient-derived organoids typically form and proliferate within the LunaX CoreMatrix dome over 3–14 days, depending on tumour type and the intended experimental endpoint. Successful PDOs appear as rounded, often lobulated cell clusters that increase in size over the culture period and can be monitored directly within the dome by brightfield microscopy.

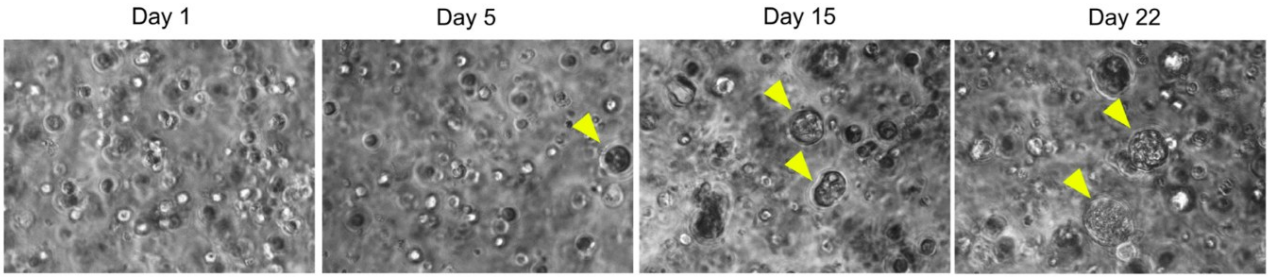

Figure 1. *Representative brightfield image of patient-derived organoids formed (yellow arrows) within LunaX CoreMatrix ECM over time. Adapted from Bock, N., et al (2023). GelMA, click-chemistry gelatin and bioprinted polyethylene glycol-based hydrogels as 3D ex vivo drug testing platforms for patient-derived breast cancer organoids. Pharmaceutics, 15(1), Article 261.*

Materials and Equipment

Reagent / Item	Specification / Concentration
LunaX CoreMatrix Low Stiffness	Gelomics Cat. No. SKU0007; includes photoinitiator
LunaX Crosslinker	Gelomics Cat. No. SKU0028
Sterile PBS	pH 7.4, 1x, sterile
Organoid Medium	As per LX-APR-001 or your protocol
Primary tumour-derived cells	Isolated and expanded according to LX-APR-001

Reagent / Item	Specification / Concentration
CO_2 incubator	37 °C, 5% CO_2, humidified
Water bath	Set at 37 °C
Benchtop centrifuge	Swing-bucket rotor
Automated or manual cell counter	-
24-well plate	Clear, flat bottom
Calibrated pipettes and sterile tips	Low-retention, wide-bore recommended

Procedure

1. Preparation

1. Warm the 1.5x LunaX CoreMatrix ECM solution to 37 °C in a water bath for 15 minutes.
2. Determine the volume of precursor solution required. Precursor solution can be prepared in 1.5 mL increments as follows:
   - Reconstitute one 2.7 mg vial of photoinitiator in 0.6 mL PBS. Protect from light.
   - In a sterile tube, combine 1 mL of 1.5x LunaX ECM solution with 0.5 mL of photoinitiator solution.
3. Reconstitute the required number of photoinitiator vials using 0.6 mL sterile PBS per vial. Protect from light.
4. Prepare the required volume of gel precursor solution by mixing the 1.5x LunaX ECM solution with photoinitiator solution as described above. Place the precursor solution in a water bath at 37 °C.
5. If you use part of a vial of photoinitiator, the remaining reconstituted photoinitiator can be stored at 2–8 °C, protected from light, for use on the same day. Only use photoinitiator solutions prepared on the day of use. For experiments on subsequent days, prepare a fresh photoinitiator solution.

2. Harvest cells

1. Harvest cells according to your standard protocol, or LX-APR-001. After enzymatic detachment, neutralise trypsin with Organoid Medium and wash the cell suspension twice with Organoid Medium or PBS (300 × *g*, 5 minutes per wash) to ensure complete removal of residual trypsin or proteases. Residual enzymes will compromise hydrogel integrity.
2. Calculate the total number of cells required based on intended hydrogel volume and target cell density. As a starting recommendation, use 1×10^6 cells/mL, equivalent to 3×10^4 cells per 30 µL dome. Prepare ~10% excess to allow for handling losses. Pellet cells and carefully remove all supernatant before resuspending in ECM.

> ***Example:*** *24 domes × 30 µL = 720 µL; adding 10% excess → 792 µL ≈ 0.8 mL. At 1×10^6 cells/mL, prepare 8×10^5 cells (800,000 cells).*

3. Encapsulation

1. Remove the warmed LunaX ECM from the water bath. Mix gently by pipetting to ensure homogeneity. Avoid introducing air bubbles.
2. Resuspend the cell pellet in precursor solution to achieve a target density of 1×10^6 cells/mL (or your alternative target density).
3. Mix gently and thoroughly using reverse pipetting to obtain a homogeneous, bubble-free suspension.

4. Dispense 30 µL domes of the cell-laden ECM into wells of the 24-well plate.

4. Photocrosslinking

1. Carefully place the plate inside the LunaX Crosslinker, ensuring the domes are not disturbed.
2. Crosslink at 10 mW/cm² for 60–90 seconds to achieve a stiffness of 0.5–1.5 kPa.

5. Culture and Maintenance

1. Immediately add 1 mL of pre-warmed organoid medium to each crosslinked dome.
2. Incubate at 37 °C, 5% CO_2 in a humidified incubator.
3. Replace medium every 2–3 days without disturbing the domes.
4. Culture PDOs for 3–14 days according to your experimental endpoint.

6. Cell Recovery (Optional)

For downstream molecular or cellular analyses, hydrogels may be enzymatically dissolved using LunaX Cell Recovery reagent according to the manufacturer's instructions. Handle gently to maintain cell viability and structural integrity.

Troubleshooting

Problem	Solution
LunaX ECM solidifies during protocol	Return ECM (with or without cells) to the 37 °C water bath until re-liquefied. Mix by gentle pipetting. Do not heat above 37 °C.
Air bubbles in the LunaX ECM suspension	Centrifuge the suspension (with or without cells) at 300 × *g* for 1 minute. If cells have been added, gently mix by pipetting to resuspend the cells.
LunaX ECM does not crosslink on light exposure	Confirm the photoinitiator was prepared fresh and reconstituted correctly.
PDOs do not form or proliferate	Confirm cell viability and use freshly prepared organoid medium. Optimise seeding density, medium formulation and culture duration according to tumour type.
LunaX ECM domes dissolve after encapsulation	Residual trypsin or protease is degrading the hydrogel. Ensure complete removal by washing the cell pellet with medium or PBS before encapsulation.
Cell outgrowth on the well surface around the gel dome	Use a non-tissue-culture-treated well plate where possible to minimise cell attachment and growth on the well surface.

References

- LunaX ECM and LunaX Crosslinker manufacturer's instructions (Gelomics)
- LX-APR-001 – Isolation and Expansion of Primary Tumour Cells from Biopsies (Gelomics)
- LX-APR-006 – Whole Mount Immunofluorescence (Gelomics).