gelomics
3D cell culture technologies

Application Note

LunaGel™ - Low Stiffness

Photocrosslinkable extracellular matrix
Cold Water Fish Gelatin

Scan to see the LunaGel 3D cell culture video protocol

Description

LunaGel™ is a photocrosslinkable extracellular matrix (ECM) based on chemically-modified pharmaceutical grade gelatin. The major components of LunaGel™ include ECM proteins collagen type I, II, IV, and V, as well as connective tissue glycoproteins and proteoglycans. LunaGel™ retains the intrinsic cell-instructive bioactivity of natural ECMs, facilitating cell attachment, proliferation, differentiation, migration, and proteolytic degradation. LunaGel's unique photocrosslinking technology allows unprecedented control over matrix porosity and stiffness, allowing researchers to replicate the physicochemical properties of a variety of healthy and diseased tissues in 3D cell culture applications.

Application

LunaGel™ is suitable for the culture of most mammalian cell types. The mechanical properties of the LunaGel™ ECM can be controlled by varying the photocrosslinking time in the Luna Crosslinker™ between 1 – 10 minutes.

Kit contents and general notes

The LunaGel™ kit contains two components:

- 5 ml LunaGel™ ECM solution supplied as a sterile 2X stock solution in PBS
- 5 vials of lyophilized photoinitiator

The mechanical properties of LunaGel™ ECM vary depending on the type of tissue culture plastic and the volume used. We recommend optimizing the mechanical properties to suit your cell type. Try 2, 4, and 8 min exposure and observe cell growth under a microscope after 5 – 7 days.

The optimal cell concentration will depend on the type of cells being cultured and the objectives of the cell culture study. As a starting point, we recommend 500,000 cells per ml of LunaGel™ ECM for spheroid forming cell types, 1 – 5 million cells per ml for stromal and mesenchymal cell types, and 6-8 million cells per ml for endothelial cell tube formation assays.

Storage

The LunaGel™ kit should be stored at 4 – 8 °C, protected from light. The shelf life of LunaGel™ is 12 months, or as indicated on the package. Following reconstitution in buffer, store the photoinitiator solution at 4 – 8 °C protected from light, and use within 7 days.

Required materials and devices

- Phosphate-buffered saline (PBS), pH 7.4
- LunaCrosslinker™
- Optional: Non-tissue culture treated polystyrene plates

gelomics
3D cell culture technologies

Experimental procedure for a final volume of 1 mL of cell-laden ECM*

*adjust volumes as required. Preparing lower volumes makes handling more challenging.

1. Lift and count cells according to your standard protocol. Inhibit and remove trypsin/protease solution from the cells.
2. Reconstitute one vial of LunaGel™ Photoinitiator with 1 ml PBS and store protected from light.
3. Transfer the required number of cells into a fresh reaction tube and pellet by centrifugation. Remove the entire supernatant, taking care to minimize liquid residues that may dilute the ECM solution in later steps.
4. Gently mix the LunaGel™ ECM solution by pipetting up and down.
5. Add 500 µl of the LunaGel™ ECM solution to the cell pellet and gently pipette up and down to resuspend cells.
6. Add 500 µl of the Photoinitiator solution to the cell suspension. Store the remaining photoinitiator solution at 4 – 8 °C, protected from light, for future use.
7. Mix thoroughly by pipetting up and down to ensure a homogenous cell suspension. Take care to avoid the introduction of air bubbles.
8. Plate the mixture in a culture dish of your choice. You can use multiwell plates (0.3 ml for 24- well, 0.15 ml for 48- well, or 0.06 ml for 96- well) or glass-bottom cell culture chambers (ideal for imaging). We recommend using non-treated culture plasticware to minimize cell adherence to tissue culture plastic.

   Pro tip: Use reverse pipetting to avoid introducing air bubbles when dispensing the mixture into well plates.
9. Crosslink the cell-laden LunaGel™ ECM by light exposure using the Luna Crosslinker™.
10. Add sufficient cell culture medium to cover the gel and incubate in a tissue culture incubator.
11. Change the cell culture medium as required, taking care not to damage the gel samples.

Troubleshooting guide

Problem	Solution
Air bubbles in LunaGel™ ECM solution.	Centrifuge solution (with or without cells) at 300*g* for 1 min and mix by pipetting up and down.
The LunaGel™ ECM solution does not crosslink when exposed to light.	Ensure photoinitiator solution is prepared fresh and/or extend crosslinking time.
Cells are not viable.	Reduce crosslinking time below 10 min.
LunaGel™ ECM samples dissolve following cell encapsulation.	Traces of trypsin or other proteases may degrade the hydrogel samples. Ensure complete removal of trypsin before cell encapsulation by washing the cells pellet with medium of buffer.

Please contact us at info@gelomics.com for questions or more information.