Product Data Sheet

Catalogue Number: SKU0009

Revision: 1



LunaGel™ Photocrosslinkable Extracellular Matrix

Bovine Bone Gelatin, High Stiffness (SKU0009)

For Research Use Only. Not for use in human or animal therapeutic or diagnostic procedures.

Product Description

 $Luna Gel^{\tiny{\text{\scriptsize IM}}} \ is \ a \ ready-to-use \ photocrosslinkable \ extracellular$ 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 musculoskeletal 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 a simple 3D cell culture format. LunaGel™ Bovine Bone Gelatin creates optically transparent hydrogels which are stable are room temperature and compatible with standard imaging systems and bioassays. Viable cells, organoids, and spheroids cultured in gelatin based Luna $\mathrm{Gel}^{\mathrm{Im}}$ ECMs can be easily harvested using a LunaGel™ Cell Recovery Kit.

Product Specification

| Option | High stiffness (0 - 25 kPa), 7.5ml |
|----------------|--|
| Kit Contents | 5 ml LunaGel™ ECM solution supplied as a sterile 1.5X stock solution in PBS 5 vials of lyophilized photoinitiator |
| Use | 3D cell culture or musculoskeletal cell types; tissue engineering; bioprinting |
| Formulation | Contains ECM proteins collagen type I, III, IV, and V, as well as connective tissue glycoproteins and proteoglycans. No active growth factors present. |
| Physical State | Supplied as solution |
| рН | 6.5 - 7.5 |
| Cell Recovery | Use LunaGel™ Cell Recovery Kit |
| Storage | Stored at 4 – 8 °C, protected from light. Ships at ambient temperature. |
| Expiry | 12 months from the manufacture date Following reconstitution in buffer, store the photoinitiator solution at 4 – 8 °C protected from light, and use within 7 days. |

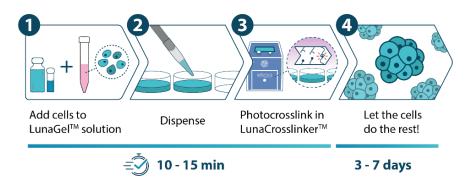
3D Cell Culture Workflow

LunaGel™ uses visible light polymerization to create 3D cell culture models with



■ ¥ 15 ■ Scan to see the LunaGel™ 3D cell culture video protocol

physiological stiffness within just minutes. Cells are resuspended in the LunaGel™ precursor solutions, dispensed into standard well plates, and cured to form stable hydrogels by exposure cell-friendly visible light LunaCrosslinker™.



Related Products

LunaCrosslinker™ (SKU 0004) – Visible Light Photocrosslinking Device LunaGel™ Bovine Bone Low Stiffness Kit (SKU 0005) LunaGel™ Cell Recovery Kit (SKU 0015)

Links

LunaGel Safety Data Sheet (SDS) Full protocol and video demonstrations

3D Cell Culture Protocol

General Notes

The LunaGel™ ECM based on mammalian gelatin (bovine or porcine) may undergo reversible thermal gelation at temperatures below ~30-°C. Heating the ECM solution to 37-°C will liquify the solution in preparation for cell encapsulation.

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, respectively, and observe cell growth under a microscope after 5 - 7 days.

The optimal cell concentration is cell-type dependent. As a starting point, we recommend 100,000 - 500,000 cells per ml of LunaGel^T 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.

Product Data Sheet

Catalogue Number: SKU0009

Revision: 1

gelomics 3D cell culture technologies

Required materials and devices

- LunaCrosslinker™ Visible Light Photocrosslinking Device
- Phosphate-buffered saline (PBS), pH 7.4
- Non-tissue culture treated polystyrene plates

Experimental procedure for a final volume of 750 µl of cell-laden ECM*

*Adjust volumes as required. Preparing lower volume < 500 µl makes handling more challenging.

- 1. Place the 1.5X LunaGel™ ECM solution into a water bath at 37 °C for approximately 15 min or until liquid.
- 2. Reconstitute one vial of LunaGel™ Photoinitiator with 500 µl PBS and store protected from light.
- 3. Lift and count cells according to your standard protocol. Inhibit and remove trypsin/protease solution from the cells.
- 4. 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.
- 5. Remove the LunaGel™ ECM solution from the water bath and mix thoroughly by pipetting up and down. Take care not to introduce air bubbles, LunaGel™ High Stiffness may be somewhat viscous consider using a positive displacement pipette for most accurate results.
- 6. Add 500 µl of the LunaGel™ ECM solution to the cell pellet and gently pipette up and down to resuspend cells.
- 7. Add 250 µl of the photoinitiator solution to the cell suspension. Store the remaining photoinitiator solution at 4 8 °C, protected from light, for future use
- 8. Mix thoroughly by pipetting up and down to ensure a homogenous cell suspension. Take care to avoid the introduction of air bubbles.
- 9. Plate the mixture in a culture dish of your choice. You can use multiwell plates or glass-bottom cell culture chambers (ideal for imaging). We recommend using non-treated culture plasticware to minimize cell adherence to tissue culture plastic. The recommended hydrogel volume for well plates is listed below.

Pro tip: Use reverse pipetting to avoid introducing air bubbles when dispensing the mixture into well plates.

| Multiwell type | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate | 384 well plate |
|-----------------|--------------|---------------|---------------|---------------|---------------|----------------|
| Volume per well | 1,200 µl | 600 µl | 300 µl | 150 µl | 50 µl | 10 µl |

- 10. Crosslink the cell-laden LunaGel™ ECM by light exposure using the Luna Crosslinker™.
- 11. Add sufficient cell culture medium to cover the gel and incubate in a tissue culture incubator. The recommended cell culture media volumes are listed below.

| Multiwell type | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate | 384 well plate |
|-----------------|--------------|---------------|---------------|---------------|---------------|----------------|
| Volume per well | 1,200 µl | 600 µl | 300 µl | 150 µl | 50 µl | 10 μΙ |

12. Change the cell culture medium as required, taking care not to damage the gel samples.

Troubleshooting Guide

| Problem | Solution |
|---|--|
| LunaGel™ ECM solution solidifies during protocol | LunaGel™ ECM solution solidifies during protocol |
| Air bubbles in LunaGel™ ECM solution. | Centrifuge solution (with or without cells) at 300g 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. |
| 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 <u>info@gelomics.com</u> for questions or more information.

