Product Data Sheet

Catalogue Number: SKU0018

Revision: 1.2



LunaGel™ Ultrapure GelMA Photocrosslinkable Extracellular Matrix (ECM)

Porcine Skin Gelatin, Type A, High Stiffness (SKU0018)



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

Product Description

LunaGel™ Ultrapure GelMA (gelatin methacryloyl) is a highly purified photocrosslinkable extracellular matrix (ECM) based on Rousselot X-Pure® GelMA that allows unprecedented control over matrix porosity and stiffness in 3D cell culture applications. Researchers can replicate the physicochemical properties of a variety of healthy and diseased tissues in a simple 3D cell culture format, utilising this ultrapure, highly consistent ECM material. LunaGel™ Ultrapure GelMA ECM creates optically transparent hydrogels which are stable at room temperature and compatible with standard imaging systems and bioassays.

Endotoxins can cause strong inflammatory responses in immune cells such as macrophages, alter their interaction with other cell types, and negatively impact the reliability of therapeutic prediction in 3D cell culture applications. Overcoming this issue, X-Pure® GelMA possesses ultra-low impurity levels (Lipopolysaccharide content: < 10 EU/g) and excellent batch-to-batch consistency, providing a superior solution for sensitive 3d cell culture applications and translational research¹.

Viable cells, organoids, and spheroids cultured in gelatin based LunaGel™ photocrosslinkable extracellular matrix (ECMs) can be easily harvested using a LunaGel™ Cell Recovery Kit (SKU0015).

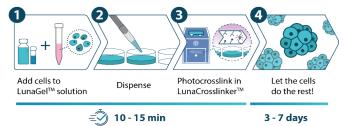
3D Cell Culture Workflow

LunaGel™ Ultrapure GelMA uses visible light polymerization to create 3D cell culture models with physiological stiffness within just minutes. Cells are resuspended in the LunaGel™ Ultrapure GelMA precursor solution, dispensed into standard well plates, and cured to form stable hydrogels by exposure to cell-friendly visible light in the LunaCrosslinker™.



Product Specification

Option	High stiffness (0 - 25 kPa), 7.5ml	
Kit Contents	5 ml LunaGel™ Ultrapure GelMA ECM solution supplied as a sterile 1.5x stock solution in PBS	
	5 vials of lyophilized photoinitiator, sterile.	
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 (SKU0015).	
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.	



Related Products

- \bullet LunaCrosslinker $^{\text{TM}}$ (SKU0004) Visible Light Photocrosslinking System
- \bullet LunaGel $^{\text{TM}}$ Ultrapure GelMA Photocrosslinkable ECM, Low Stiffness Kit (SKU0017)
- LunaGel™ Cell Recovery Kit (SKU0015)

Links

<u>LunaGel™ Safety Data Sheet (SDS)</u>
Full protocol and video demonstrations

3D Cell Culture Protocol

General Notes

LunaGel™ Ultrapure GelMA ECM is based on mammalian gelatin and 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™ Ultrapure GelMA 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™ Ultrapure GelMA 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.

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Required Materials and Devices

- LunaCrosslinker™ Visible Light Photocrosslinking Device
- Phosphate-buffered saline (PBS), pH 7.4
- Non-tissue culture treated polystyrene well 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™ Ultrapure GelMA 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 LunaGel™ Ultrapure GelMA ECM solution in later steps.
- 5. Remove the LunaGel™ Ultrapure GelMA ECM solution from the water bath and mix thoroughly by pipetting up and down. Take care not to introduce air bubbles, LunaGel™ Ultrapure GelMA High Stiffness may be somewhat viscous consider using a positive displacement pipette for most accurate results.
- 6. Add 500 µl of the LunaGel™ Ultrapure GelMA 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™ Ultrapure GelMA ECM by light exposure using the LunaCrosslinker™.
- 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 μΙ	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™ Ultrapure GelMA ECM solution solidifies during protocol	Re-heat LunaGel™ Ultrapure GelMA ECM solution (with or without cells) to 37 °C in a water bath. Once liquid, mix by pipetting up and down. Do not heat above 37 °C.
Air bubbles in LunaGel™ Ultrapure GelMA ECM solution.	Centrifuge solution (with or without cells) at 300g for 1 min and mix by pipetting up and down.
The LunaGel™ Ultrapure GelMA 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™ Ultrapure GelMA 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.

¹ Heinrich, M.A., et al. (2023), Endotoxin contamination alters macrophage-cancer cell interaction and therapeutic efficacy in pre-clinical 3D in vitro models, Biomaterials Advances, Volume 144, 213220; https://doi.org/10.1016/j.bioadv.2022.213220.

