Ref No: BPR-5423-1KIT
Date: 25-MAR-2026
Author: CS, PG, EP, VK. Version: 1

CELLINK
A BICO COMPANY

BIOPRINTING PROTOCOL

GelMA 95% DS

This is a suggested procedure; please adjust according to your experimental needs. Always keep the photoink and all its components protected from light.

Protocol aim

The aim of this protocol is to provide step-by-step instructions to prepare the GelMA 95% DS photoink from stock components and print it on the LUMEN X Gen 3 using recommended starting parameters.

Materials needed

- LUMEN X Gen 3
- GelMA 95% DS Stock Solution*
- LUMEN X Vat (clean, empty)*
- LUMEN X Glass Build Platform (clean)*
- Xcite Stock Solution*
- Xsorb Stock Solution*
- PBS (Phosphate Buffered Saline, sterile)
- Nitrile gloves and lab coat
- Chemical safety goggles
- Water bath or heating block set to 37°C
- 3 mL syringe + compatible nozzle/needle or positive displacement pipette
- Calibrated pipettes and tips (0.1 mL accuracy)
- 1 and 15 mL amber Eppendorf tubes or light-protected containers
- USB drive with STL files of models intended for printing
- Razor blade or scalpel
- Deionized water
- Ethanol
- Fume-dispensing wash bottle containing 70% isopropanol
- Compressed air (optional)
- Spirit/bubble level and magnetic vises

*Components available individually or as the GelMA 95% Photoink Kit (includes 2×10 mL GelMA 95%, Xcite, Xsorb, amber tubes, and plastic razor blades). See www.cellink.com/product/gelma-95/

1. Safety and preparation

- Review the GelMA 95% DS SDS before handling. CLP classification: None (not classified as hazardous). Standard laboratory PPE is sufficient.
- Wear nitrile gloves and lab coat. Wear chemical safety goggles where eye exposure is reasonably probable.
- GelMA 95% DS Stock Solution, Xcite, and Xsorb are light-sensitive. Keep vials sealed and refrigerated (2-10 °C) until immediately before use.
- Ensure vat heating target temperature is 37 °C; do not exceed 40 °C during printing.
- Do not let GelMA 95% DS residue enter drains. Dispose of according to local regulations.

2. Photoink formulation

2.1 Acellular printing

- Place the GelMA 95% DS Stock Solution vial in a water bath or heating block at 37 °C for at least 15 min until fully liquefied. The solution must be liquid for accurate pipetting – do not pipette cold.
- Allow Xcite and Xsorb to reach room temperature (≥10 min before opening).
- Using positive displacement calibrated pipettes, add to a 15 mL amber tube in this order: GelMA 95% DS → PBS → Xcite → Xsorb (see **Table 1**). Scale up/down proportionally for larger/smaller volumes.
- Work quickly while GelMA is still liquid (above ~25 °C). If the solution begins to gel during mixing, briefly warm to 37 °C again.
- Cap tightly and homogenize by inverting 20 times. The solution should appear visually uniform.
- Allow bubbles to settle fully while warming (5-10 min). Do not load if phase separation or large bubbles are visible.
- Use the photoink promptly after formulation is prepared. If stored, keep refrigerated and light-protected for up to 7 days. Label with preparation date. Re-warm to 37 °C before re-using.

⚠ Work quickly while GelMA is liquid (above ~25 °C). If the solution begins to gel during mixing, briefly re-warm to 37 °C before continuing.

Table 1. GelMA 95% DS recipe intended for 15 mL of photoink at 10% GelMA concentration.

Component	Volume
GelMA 95% DS Stock Solution	10.000 mL
Xcite	1.875 mL
Xsorb	0.1875 mL
PBS	2.9375 mL

2.2. Cellular printing

For bioprinting with live cells, the formulations were tested with 0.5×10^6 cells/mL (see **Table 2**). For printing with much higher cell densities, adjustments to the component's ratios might be necessary. Keep all components warm at 37 °C throughout the workflow.

Mixing technique for 7.5% and 10% GelMA:

- Pre-warm GelMA 95% DS to 37 °C.
- Combine Xcite + Xsorb + Cell media into a single "master liquid" (0.500 mL at 7.5%; 0.333 mL at 10%).
- Resuspend the dry cell pellet in this master liquid, then gently add the cell suspension to the warmed GelMA.

Mixing technique for 12.5% GelMA:

- The cell media volume (0.030 mL) is too small for reliable cell resuspension. Instead: pre-warm GelMA 95% DS to 37 °C and combine with cell media (total vehicle = 0.863 mL).
- Resuspend the dry cell pellet in this GelMA + media mixture.

- Add Xcite + Xsorb (0.137 mL total) to the cell-laden GelMA and homogenize gently.
- Keep at 37 °C and load promptly.
- Xcite and Xsorb scaling: All three formulations (7.5%, 10%, 12.5%) use the same absolute Xcite (0.125 mL) and Xsorb (0.012 mL) volumes. This is an intentional decision to limit photoinitiator concentration and protect cell viability across all concentrations. As a result, the Xcite:GelMA ratio decreases with increasing GelMA concentration (7.5%: 0.250; 10%: 0.187; 12.5%: 0.150), which is compensated by the corresponding reduction in exposure time at higher concentrations (see **Table 3**).

Table 2. GelMA 95% DS cell-laden recipes intended for 1 mL of photoink at three GelMA concentrations.

Component	**7.5% GelMA (1 mL)**	**10% GelMA (1 mL)**	**12.5% GelMA (1 mL)**	**Notes**
GelMA 95% DS Stock Solution (mL)	0.500	0.667	0.833	Pre-warm to 37°C before pipetting
Xcite (mL)	**0.125**	**0.125**	**0.125**	
Xsorb (mL)	**0.012**	**0.012**	**0.012**	Add last; homogenize well
Cell culture media (mL)	0.363	0.196	0.030	Use to resuspend cell pellet
Total volume (mL)	1.000	1.000	1.000	

Notes:

- For faster reactivity: increase the proportion of Xcite.
- For finer spatial resolution: increase the proportion of Xsorb.
- For softer mechanical properties: decrease GelMA 95% DS proportion and/or replace some stock with a long-chain polymer solution.
- For stiffer mechanical properties: increase GelMA 95% DS proportion and/or replace some stock with a short-chain polymer solution.
- For enhancement of biological/mechanical properties (growth factors, polymers, etc.): replace stock solution or PBS with a solution of the target substance and/or add additional powders.

3. File setup (import model)

- Insert the USB drive into the LUMEN X front USB port or open DNA Studio Illuminate on your computer.
- Confirm that the LUMEN X is connected to DNA Studio Illuminate by clicking the Connection button at the top of the main screen (local connection).
- On the LUMEN X display: Home → New Print Protocol → select Model.
- Select between (small, medium, or large) Build Platform as the print surface according to the size of your model → press Next.
- In the Geometry screen, press Import; navigate to the USB and open the desired STL or OBJ file.

4. Set print parameters

- Set Layer Height, Exposure, and Build Platform Adhesion per **Table 3** below.
- Set Light Intensity per **Table 3**. On a calibrated LUMEN X Gen 3, ≈20 mW/cm² corresponds to approximately 70% in the UI. For 20 µm layer height, reduce light intensity to ≈16 mW/cm² (approximately 56% in the UI) as indicated in **Table 3**. Verify calibration within the last 6 months.
- Enable Build Platform Adhesion using the recommended build platform adhesion value (starting point: 10 s).
- Use transition layers when the difference between the build-platform adhesion exposure time and the layer exposure time is greater than the exposure time itself. Set the transition-layer exposure time to an intermediate value, typically the average of the adhesion exposure time and the exposure time.
- Enable vat heating and set the target temperature to 37 °C. Do not exceed 40 °C during printing.

Table 3. Recommended print parameters for GelMA 95% DS Stock Solution on LUMEN X Gen 3.

Layer thickness (µm)	Exposure (s)			Build platform adhesion (s)	Power (mW/cm²)
	7.5% GelMA	10% GelMA	12.5% GelMA		
100	10.0	8.0	7.5	10.0	≈ 20
50	7.0	5.5	5.0	10.0	≈ 20
20	5.0	4.0	3.5	10.0	≈ 20

*⚠ 20 µm using **Table 3** settings is not recommended for cell-laden prints. Use 50 µm or 100 µm for cell-laden bioprinting applications to increase cellular viability.*

⚠ Always validate a single, centered part before printing arrays. Stray light from adjacent features can cause over-curing and dimensional inaccuracies. Confirm single-part quality first, then increase inter-part spacing incrementally if required.

5. Slice and start

- Press Next to move to Slicing.
- Press Slice and wait until the slicing process is complete.
- Once completed, press Next again.
- On the Summary page, verify all settings, then press Go to Print.

6. Build platform leveling calibration (auto-levelling)

- When prompted, select Yes to start calibration; the print arm will “home”.
- When prompted, insert the clean, empty vat and press Yes. Use the magnetic vises and a spirit level to verify that the vat is correctly installed and free of tilt.
- When prompted, insert the Glass Build Platform. Do not tighten the levelling screw yet and ensure that the build platform is correctly aligned and installed.
- When prompted, make sure the build platform levelling screw is unlocked, then press Yes to begin auto-levelling.
- When prompted, tighten the levelling screw and confirm that it is locked. The build arm will then rise.

⚠ A loose levelling screw is the most frequent cause of model detachment. Verify the screw is fully tightened before proceeding.

7. Photoink preparation and loading

- Re-warm the photoink to 37°C immediately before loading if it has cooled and gelled.
- Homogenize immediately before loading (invert 5-10 times).
- Take a pipette or attach a nozzle to an empty 3 mL syringe. Load the recommended volume of GelMA 95% DS photoink prompted in the UI and deposit into the center of the vat.
- Use a fume-dispensing wash bottle containing 70% isopropanol to burst any remaining bubbles, ensure that only small amounts of IPA vapor are dispensed. Work quickly - GelMA will begin to gel as it cools in the vat.
- On the display, press Confirm, material added.

⚠ Do not confirm material loaded if bubbles are visible on the vat window. Remove them with a syringe or IPA vapor before continuing.

⚠ GelMA gels as it cools in the vat. Start printing without delay after confirming material loaded.

8. Print execution

- Press Start Print.
- Never reuse excess GelMA from a previous print. Fully clean the vat before starting a new print.
- Close the enclosure door during printing.
- At print completion, the front LED strip turns green.
- Restart the print if required or save the protocol for later use to repeat the print with the same settings and image slicing included.

9. Post-print inspection

- Remove the magnetic build platform carefully. Inspect the print:
    - The model is attached to the build platform.
    - Ensure that the lattice is centered on the build platform.
    - Model is fully printed - no missing sections.
    - Layers are intact - no splitting or delamination.

10. Print removal and cleaning

- Using a plastic razor blade or a scalpel, gently slide under the model corners and slowly lift free.
- Vat: wash with deionized water (do NOT use IPA). Air dry - do not use paper towels as it can leave debris or fibers on the vat surface.
- Build platform: spray with ethanol, rinse with deionized water, air dry.
- GelMA 95% DS residue: do not let enter drains. Dispose of according to local regulations and institutional waste disposal guidelines.

11. Documentation

- When the print is complete, the dialog box displays “Print complete” with a green checkmark. Optionally press Add print comments to annotate the run. Press Save protocol to save the record; press Close protocol to exit or Restart print to repeat.
- Record batch/lot numbers for GelMA 95% DS, Xcite, and Xsorb, plus the photoink preparation date and heating duration.