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CELLINK
A BICO COMPANY

PRINTING PROTOCOL

PEGDA Soft

This is a suggested procedure; please adjust according to your experimental needs. PEGDA Soft Stock Solution is classified as hazardous (H315/H317/H318). Always wear chemical splash-proof goggles and nitrile gloves. Minimize ambient light exposure.

Protocol aim

The aim of this protocol is to provide step-by-step instructions to prepare the PEGDA Soft photoink from stock components and print it using LUMEN X Gen 3 with recommended starting parameters.

Materials needed

- LUMEN X Gen 3
- PEGDA Soft Stock Solution*
- LUMEN X vat (clean, empty)*
- LUMEN X Glass Build Platform (clean)*
- Xcite Stock Solution*
- Xsorb Stock Solution*
- PBS (Phosphate Buffered Saline, sterile)
- Chemical splash-proof goggles
- Nitrile gloves and lab coat
- 3 mL syringe + compatible nozzle/needle or positive displacement pipette
- Calibrated pipettes and tips (0.1 mL accuracy)
- 1 and 20 mL amber Eppendorf tubes or light-protected containers
- USB drive containing STL files of models
- Razor blade or scalpel
- Deionized water
- Ethanol
- Compressed air (optional)
- Spirit/bubble level and magnetic vises

*Components available individually or as the PEGDA Soft Photoink Kit — see www.cellink.com/product/pegda-soft/

1. Safety and preparation

- Review the PEGDA Soft SDS before handling. Signal word: DANGER - H315 skin irritation, H317 allergic skin reaction, H318 serious eye damage.
- MANDATORY PPE: chemical splash-proof goggles (not standard safety glasses), nitrile gloves, and lab coat. Replace gloves immediately if torn.
- All three components (PEGDA Soft, Xcite, Xsorb) are light-sensitive. Keep vials sealed and refrigerated until immediately before use.
- Ensure printbed temperature control is OFF; do not exceed 30 °C.
- First aid: skin contact - wash immediately with soap and water; eye contact - rinse with water for ≥15 min and call a doctor.

2. Photoink formulation

- Work under reduced ambient light using an amber or foil-wrapped tube. Allow PEGDA Soft, Xcite, and Xsorb to reach room temperature (≥10 min before opening).
- Using calibrated pipettes, add the following components to a 20 mL amber tube in this order to get 20 mL of photoink (see **Table 1**): PEGDA Soft (10.00 mL) → PBS (7.25 mL) → Xcite (2.50 mL) → Xsorb (0.25 mL). Add Xsorb last to facilitate better dispersion. Scale proportionally for larger volumes.
- Cap tightly and homogenize by vigorously inverting 20 times. The solution should appear visually uniform.
- Allow bubbles to settle fully (5–10 min). Do not load if phase separation or large bubbles are visible.
- Formulated photoink can be stored refrigerated and light-protected for up to 7 days.

Table 1. PEGDA Soft photoink recipe intended for 20 mL of photoink.

Component	Volume (mL)
PEGDA Soft Stock Solution	10.00
Xcite	2.50
Xsorb	0.25
PBS	7.25

Notes:

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

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 (next to Geometries).
- Navigate to the USB drive and open the desired STL or OBJ.

4. Set print parameters

- Set Layer Height, Exposure, and Build Platform Adhesion per **Table 2** below.
- Set Light Intensity per Table 2 (≈70% on a calibrated LUMEN X Gen 3 ≈ 20 mW/cm²). Verify calibration within the last 6 months.
- Enable Build Platform Adhesion using the recommended adhesion value (starting point: 14 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.
- Use the parameters below as a starting point. Note: PEGDA Soft parameters differ from PEGDA X - do not change them.

Table 2. Recommended starting print parameters for PEGDA Soft on LUMEN X Gen 3.

Layer thickness (µm)	Exposure (s)	Transition layer section (s)	Build platform adhesion (s)	Power (mW/cm²)
100	11	N/A	14	≈ 20
50	7	N/A	14	≈ 20
20	5	9.5	14	≈ 20

⚠ At 20 µm: add a 9.5 s transition layer between the build platform adhesion layer and the standard exposure layers.

⚠ 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 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. Optionally use 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

- Take a pipette or attach a nozzle to an empty 3 mL syringe.
- Homogenize the vial with a photoink before printing. Load the recommended volume of PEGDA Soft prompted in the UI and deposit into the center of the vat.
- If small bubbles remain after loading, gently burst them using a pipette tip, taking care not to disturb the material surface.
- 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.

8. Print execution

- Press Start Print.
- Never reuse excess PEGDA Soft 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 the print free.
- Clean the vat by washing it with deionized water only. Do not use IPA. Leave it to air dry and do not use paper towels as it can leave debris or fibers on the vat surface.
- Clean the build platform by spraying it with ethanol, rinsing it with deionized water, and then allowing it to air dry or drying it with compressed air. Do not use paper towels.
- Do not dispose of PEGDA Soft residue down the sink or allow it to enter drains. Dispose of it 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 PEGDA Soft, Xcite, and Xsorb, plus the photoink preparation date.