

Usage Protocol

GelMA PhotoInk™

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to explain to first time users how to print with Volumetric's GelMA PhotoInk. The instructions are written for use with the Lumen X™ bioprinter and assumes the user has the system and accessories. This document covers pre-print setup, mixing with cells, 3D bioprinting and post-print processes of removing and washing the print. Adding high concentrations of particles or cells to the PhotoInk will increase the photocrosslinking time.

Storage

- Recommended storage temperature: 4 °C
- Do NOT freeze the GelMA PhotoInk.
- Keep in a dry, dark location when not in use.
- Protect from free radical initiators, light, and sources of heat.

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Material needed

- Vial of GelMA PhotoInk*
- Micropipette, 1000 µL recommended
- Box of pipette tips
- Plastic Razor Blade
- Container, 250 mL or larger, filled with either:
 - o DI water
 - o PBS or buffer of choice*
 - o Cell culture media, optional for printing with cells*
- Syringe, optional for clearing channels
- Needle, optional for clearing channels*
- Lumen X Bioprinter*

* The product can be purchased in the CELLINK store at www.cellink.com/store/.

** Note: acidic and basic buffers will degrade the printed hydrogel.

Protocol

Before Printing			
Step	Title	Material	Description

1	Preheating bioprinter	- Lumen X 3D Bioprinter	<p>Activate the Print bed heating function in LightField™.</p> <p>Although the Current temperature and Target temperature will display 60°C, thermal transfer through the glass and PDMS should result in a temperature of 37°C, depending on the room temperature.</p> <p>CAUTION: Heater glass can rapidly reach 70°C and cause pain or injury if touched!</p>
2	Preheating vat	- PDMS vat	Begin warming PDMS vat either in an incubator or by installing the vat onto the Lumen X.
3	PhotoInk Temperature	- PhotoInk Vial	Heat up PhotoInk vial until it reaches 37°C for 20 minutes in either an incubator or water bath.
4	Set up the print		Prepare STL file for printing by progressing through the File, Prepare, and Print tabs (See Lumen X manual for details).

Table 1. Recommended parameters: however, parameters written on the PhotoInk vial supersede the numbers written below.

Power (mW/cm ²)	20	
Layer Height (µm)	100	50
Exposure Time (s)	8	4.5
1 st Layer Time Scale Factor	3x	4x

Step	Title	Material	Description
5	Mix cells with PhotoInk	- Cell solution - Preheated - GelMA PhotoInk vial - Pipette and tips	<p>If not printing with cells move directly to step 6.</p> <p>Centrifuge cell solution to create a pellet.</p> <p>Aspirate and dispose of supernatant media.</p> <p>Gently pipette the volume of PhotoInk calculated in step 4 such that the cells are evenly mixed in the solution.</p>
6	Dispense material	- PhotoInk Vial - Micropipette - Pipette tips	Dispense the volume of PhotoInk displayed by LightField. Return the remaining PhotoInk to a dark place close-by if more prints will be conducted or to 4 °C for storage
8			Tap Start

After Printing			
9	Part removal	- Plastic razor	Remove the build platform from the Lumen X. Use the plastic razor blade to gently remove the printed part.
10	Washing parts	- Container filled with wash fluid	Place the print in the container of PBS or cell culture media to wash the bulk material off. Do not use pure water as it causes GelMA Photoink to swell. The PBS should be replaced at least three times within 24 hours such that the dye washes away sufficiently within a day.
11	Clearing channels	- Syringe - Needle	If there are channels, a syringe and needle can be used to perfuse the wash solution and remove uncured material.

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