

Usage Protocol

PEGDA500 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 PEGDA500 PhotoInk. The instructions are written for use with the Lumen X™ bioprinter and assumes the user has the system and accessories. The PEGDA500 PhotoInk is not formulated to be printed with cells encapsulated within the resultant hydrogel, nor to expect cell adhesion.

Storage

- Recommended storage temperature: 4 °C
- Do NOT freeze the PEGDA500 PhotoInk.
- Keep in a dry, dark location when not in use.
- Protect from free radical initiators, light, and sources of heat.

1

Material needed

- Vial of PEGDA500 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*
- 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	PhotoInk Temperature	- PhotoInk Vial	Allow PhotoInk to reach room temperature.

2	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)	4	2
1 st Layer Time Scale Factor	3x	3x

Step	Title	Material	Description
3	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
4			Tap Start
After Printing			
5	Part removal	- Plastic razor	Remove the build platform from the Lumen X. Use the plastic razor blade to gently remove the printed part.
6	Washing parts	- Container filled with wash fluid	Place the print in the container of PBS or water to wash the bulk material off. The PBS should be replaced at least three times within 24 hours such that the dye washes away sufficiently within a day.
7	Clearing channels	- Syringe - Needle	If there are channels, a syringe and needle can be used to perfuse the wash solution and remove uncured material.
9	Sterilizing parts		Note: This step is not necessary if the construct has been printed using aseptic technique in a BSC2 using sterile Photoink. The construct can be sterilized after submerging in 0.22 µm filtered 70% ethanol for 5 min, followed by submerging in sterile PBS twice, for 30 minutes each, to ensure the ethanol is washed away.