Usage Protocol

# PEGDA200 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 PEGDA200 PhotoInk. The instructions are written for use with the Lumen X™ bioprinter and assumes the user has the system and accessories. The PEGDA200 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 PEGDA200 PhotoInk.
- Keep in a dry, dark location when not in use.
- Protect from free radical initiators, light, and sources of heat.



## Material needed

- Vial of PEGDA200 PhotoInk*
- Micropipette, 1000 µL recommended
- Box of pipette tips
- Plastic Razor Blade
- Container, 250 mL or larger, filled with either:
  - DI water
  - 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/](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) | 6 | 3 |
| 1$^{st}$ Layer Time Scale Factor | 3x | 3x |

| Step | Title | Material | Description |
|---|---|---|---|
| 3 | Dispense material | - PhotoInk Vial<br>- Micropipette<br>- 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.<br><br>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.<br><br>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<br>- 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.**<br><br>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. |