



## Directions of use – freeze-dried ClaroBGI800

### Preparation of GelMA stock solution (recommended is 20 % w/v)

1. Dissolve ClaroBGI800 freeze dried GelMA in PBS solution 1X in water bath at 40°C for 3 h or until complete dissolution of the product. Swirl the flask gently within the timeframe of dissolution.
  - a. This step should be performed under sterile ambient – laminar flow cabinet
  - b. Do not vortex the solution
  - c. Dissolution time may vary according to the concentration desired

### Dilution of 20% w/v GelMA solution with 1% w/v LAP solution (prepared using sterile PBS solution)

1. Prepare a 1% w/v LAP solution stock solution in sterile PBS solution.
2. Sterile filter the LAP stock solution under sterile ambient – laminar flow cabinet
3. In a sterile container (protected from light), mix the ClaroBGI800 stock solution with the LAP stock solution at a 1:1 volume ratio
4. Close the container containing the mixture tight
5. Place the container in water bath at 40°C for additional 30 min or until complete homogenization of the two solutions. Swirl the flask gently within the timeframe of dissolution. The final solution will be at concentration 10% w/v (with 0.5% w/v LAP).
  - a. Ensure that the solution is protected from light to prevent premature photoinitiator activation.

### Cell mixing (if printing with cells)

1. Reserve 2 needle-free syringes with luer-lock connection
2. Draw up cell suspension into one the syringes and the pre-warmed ClaroBGI800-LAP solution in the second syringe.
3. Using a *Syringe-to-Syringe Coupler*, attach the syringe containing ClaroBGI800-LAP to the syringe containing the high-density cell suspension.
4. Begin mixing by transferring the cell suspension into the sterile pre-filled syringe containing the ClaroBGI800-LAP solution. Push the combined material gently back and forth 6 times until the consistency is even.
5. Once mixed, the cell composite is ready for 3D printing.

### 3D printing

1. After cell mixing (if printing with cells), allow the ClaroBGI800-LAP solution to build up the viscosity for approximately 25-35 min prior printing (honey-like consistency,



slow-moving bubble inside the printing cartridge or syringe) at ambient conditions of temperature.

2. Below are the printing parameters of ClaroBGI800-LAP hydrogel without cells using a commercially available extrusion-based printer.

Nozzle	Print head Temperature (°C)	Ambient Temperature (°C)	Time to achieve extrudable consistency (min)	Pressure (kPa)
27G	24 ± 1	20 ± 2	30 ± 2	40 ± 5

### **Crosslinking**

1. ClaroBGI800 is crosslinkable under 405 nm light in the presence of a photo initiator (e.g. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, LAP). Light dosage should be adjusted according to the desired tissue stiffness.