

## GelMA-Alginate Kit Protocol

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### Kit Components

Item	Quantity	Storage
CELLINK GelMA Powder - Sterile	1000 mg	-20 °C, protected from light
CELLINK Alginate Powder - Sterile	200 mg	Room Temperature
Nozzle Kit	1	Room Temperature
Sterile 3 cc Cartridges	3	Room Temperature
Sterile luer-lock	3	Room Temperature
Sterile 12 mL syringe	3	Room Temperature

### Materials not included

Item	Quantity
Stir Bar – Sterile	2
PBS	20 mL
50 mL Falcon Tube - Sterile	2
Sterile 0.22 µm filter	2
Irgacure 2959 or LAP	200 mg
Sterile Serological pipets	2

### Protocol Summary

This kit and protocol are intended for the generation of GelMA-Alginate bioinks for 3D bioprinting. The kit contains two components, sterile GelMA powder and sterile Alginate powder. The instructions will direct the reconstitution of a GelMA solution and an Alginate solution that finally is mixed at 1:1 ratio to generate the bioink. The components will be reconstituted at twice the final concentration to be diluted upon mixing.

### Examples of common Compositions and Recipes

GelMA-Alginate Bioink	GelMA wt% Needed	Alginate wt% Needed
5%-1.5%	10% GelMA	3%
7.5%-1.5%	15% GelMA	3%
10%-1.5%	20% GelMA	3%
5%-3%	10% GelMA	6%
7.5%-3%	15% GelMA	6%
10%-3%	20% GelMA	6%

**Alginate Precursor Solution Reconstitution Protocol**

*This alginate precursor solution will be made at twice the desired final concentration.*

1. Prepare 20 mL of PBS or your desired reconstitution buffer.
2. Sterile filter this buffer into a sterile 50 mL Falcon Tube.
3. Pipet the desired volume of the sterilized reconstitution solution to the vial of CELLINK Alginate powder to achieve the desired concentration.

<b>Final Concentration Desired</b>	<b>Volume Reconstitution Solution Needed</b>
1% (10 mg/mL)	20 mL
2% (20 mg/mL)	10 mL
3% (30 mg/mL)	6.66 mL
4% (40 mg/mL)	5 mL
5% (50 mg/mL)	4 mL

4. Add a sterile stir bar to the vial.
5. Stir solution overnight at room temperature to ensure dissolution.
6. Transfer alginate precursor solution to a syringe.

### **GelMA Precursor Solution Reconstitution Protocol**

*This GelMA precursor solution will be reconstituted with twice the desired final concentration for GelMA, since Alginate Precursor solution will be mixed in at a ration 1:1.*

1. Remove CELLINK GelMA powder from storage and return to room temperature.
2. Prepare 25 mL of warmed PBS.
3. Mix in the desired amount of photoinitiator to achieve the necessary precursor solution concentration.

<b>Final PI Concentration</b>	<b>PI mass for 25 ml of Buffer Stock</b>
0.05% (0.5 mg/mL)	12.5 mg
0.10% (1 mg/mL)	25 mg
0.25% (2.5 mg/mL)	62.5 mg

4. Sterile filter the photoinitiator solution using the 12 mL syringe and 0.22 µm sterile filter into a sterile falcon tube.
5. Heat the sterile photoinitiator solution to 60 °C.
6. Add the desired volume of heated photoinitiator solution to the vial of CELLINK GelMA powder to achieve the desired concentration.

<b>Final Concentration Desired</b>	<b>Volume Reconstitution Solution Needed</b>
5% (50 mg/mL)	20 mL
10% (100 mg/mL)	10 mL
15% (150 mg/mL)	6.66 mL
20% (200 mg/mL)	5 mL

7. Stir the mixture for 30 minutes at 70 °C to ensure dissolution.
8. Transfer GelMA precursor solution to a syringe and cover with foil to protect from light.

### **Mixing GelMA-Alginate**

1. Warm up both the GelMA and Alginate precursor solutions to 37 °C
2. Transfer to the necessary volume of each solution from the stock syringe to a new syringe using a luer-lock connector.
3. Mix the two precursor solutions using a dual-syringe mixing technique a minimum of 25 times back and forth.
4. Transfer the whole volume to one syringe and cap.
5. Lightly centrifuge (500 rpm) to remove air bubbles.
6. Transfer into 3 cc cartridge for bioprinting.