

Reconstitution Protocol

GelMA-Alginate Kit

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for reconstituting the GelMA and Alginate powders. This protocol is intended for the generation of GelMA-Alginate bioinks for 3D bioprinting and 3D cell culture. The kit contains three components, sterile GelMA powder, sterile Alginate powder and chosen photoinitiator (PI) e.g. LAP or Irgacure 2959. The instructions will direct the reconstitution of a GelMA solution and an Alginate solution that is then mixed at a 1:1 ratio to generate the bioink. The components will be reconstituted at twice the final concentration to be diluted upon mixing.

Material needed

- GelMA powder (500 mg), sterile *
- Alginate powder (100 mg), sterile*
- Sterile PBS 1X (without calcium or magnesium)
- LAP or Irgacure 2959 (100 mg)
- Optional: HEPES buffer (1 M), NaOH or HCl
- Sterile serological pipettes, 2 pcs
- Sterile stir bars, 2 pcs
- Sterile syringes
- Sterile 0.22 µm filter, 2 pcs
- Sterile Falcon Tube
- Female/female luer lock connectors*
- UV shielding cartridge, 3cc*

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

Protocol

This protocol is described for mixing of 1 vial of GelMA and 1 vial of Alginate. The Alginate and GelMA precursor solutions will be made at twice the desired final concentration since they will be mixed together 1:1, see Table 1 below for suggestion of compositions to mix up the GelMA and Alginate. Remove the GelMA powder from storage to reach room temperature.

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Table 1. Suggestions of printable compositions of GelMA and Alginate.

GelMA-Alginate Bioink	wt% GelMA precursor solution	wt% Alginate precursor solution
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

Step	Title	Material	Description
1	Prepare PBS	- Sterile PBS	- Prepare 10 ml, or as much solution as is needed based on Table 2, of PBS or your desired reconstitution solution. Note: Examples of reconstitution solution can be cell culture medium or mannitol solution.
2	Prepare Alginate solution	- Vial of Alginate - Reconstitution solution - Sterile serological pipette - Sterile stir bar - Sterile 12 mL syringe	- Using a sterile serological pipette, add the desired volume of the reconstitution solution to the vial of Alginate powder to achieve the desired concentration, see Table 2. - Add a sterile stir bar to the vial or place on shaker overnight at room temperature to ensure dissolution, - Transfer alginate precursor solution to a syringe.

Table 2. Suggestions of Alginate precursor solution concentrations for mixing one vial of 100 mg of Alginate.

Desired concentration of Alginate precursor solution	Volume Reconstitution Solution Needed
1% (10 mg/mL)	10 mL
2% (20 mg/mL)	5 mL
3% (30 mg/mL)	3.33 mL
6% (60 mg/mL)	1.66 mL

GelMA Precursor Solution Reconstitution Protocol

Step	Title	Material	Description
1	Prepare PBS and PI	<ul style="list-style-type: none"> - Sterile PBS - Optional: HEPES buffer (1 M) - PI of choice - Sterile 12 mL syringe - Sterile 15 mL Falcon tube - Sterile 0.22 μm filter 	<ul style="list-style-type: none"> - Prepare 12 mL or as much solution as is needed based on Table 4, of PBS or your desired reconstitution buffer. - Note: Examples of reconstitution buffers can be cell culture medium or mannitol solution. - It is optional to have 10 mM HEPES buffer in the reconstitution solution, if your solution does not already contain a buffer for maintaining a physiologic pH in the final bioink. - Mix in the desired amount of PI in the reconstitution solution to achieve the necessary precursor solution concentration, see Table 3. - Sterile filter the PI solution using the 12 mL syringe and 0.22 μm sterile filter into a sterile 15 mL Falcon tube. - Heat the sterile PI solution to 60°C.
2	Prepare GelMA solution	<ul style="list-style-type: none"> - Vial of GelMA - Reconstitution solution - Sterile serological pipette - Sterile stir bar - Sterile 12 mL syringe 	<ul style="list-style-type: none"> - Using a sterile serological pipette, add the desired volume of the sterilized reconstitution solution to the vial of GelMA powder to achieve the desired concentration, see Table 4. - Add a sterile stir bar to the vial. - Stir the mixture for 30 minutes at 70°C to ensure dissolution. - Transfer GelMA precursor solution to the 12 mL syringe and cover with foil to protect from light.

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Table 3. Suggestions of PI concentrations for the GelMA precursor solution.

PI Concentration in GelMA precursor solution	PI mass for 12 mL of Reconstitution solution
0.05% (0.5 mg/mL)	6 mg
0.10% (1 mg/mL)	12 mg
0.25% (2.5 mg/mL)	30 mg

Table 4. Suggestions of GelMA precursor solution concentrations for mixing one vial of 500 mg of GelMA.

Desired concentration of GelMA precursor solution	Volume Reconstitution Solution Needed
5% (50 mg/mL)	10 mL
10% (100 mg/mL)	5 mL
15% (150 mg/mL)	3.33 mL
20% (200 mg/mL)	2.5 mL

Mixing GelMA-Alginate Protocol

Step	Title	Material	Description
1	Mix GelMA and Alginate	<ul style="list-style-type: none"> - Sterile syringe - Luer lock connectors - UV shielding cartridge, 3cc - NaOH or HCl 	<ul style="list-style-type: none"> - Warm up both the GelMA and Alginate precursor solution to 37°C. - Transfer the necessary volume of each solution from the stock syringe to a new syringe using a luer-lock connector. - Connect the two syringes of GelMA and Alginate precursor solutions using a luer lock and mixing back and forth a minimum of 25 times. - Double check that the pH is between 7.0-7.5 since the pH is important for the proper viscosity of the bioink. If needed, balance with NaOH or HCl. <p>Note: Adding additional liquids to adjust the pH will dilute your bioink and PI concentration. In addition, be careful with addition of NaOH since a too high pH can start to degrade the GelMA.</p> <ul style="list-style-type: none"> - Transfer the whole volume to one syringe and cap. - Lightly centrifuge (500 rpm) to remove air bubbles - Transfer into 3cc cartridge for bioprinting. See <i>Bioprinting Protocol GelMA A</i> for an example procedure for how to print with a GelMA-Alginate blend. Adjust printing conditions depending on the chosen concentrations.