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Reconstitution Protocol

GelMA 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 power. This protocol is intended for the generation of GelMA bioinks for 3D bioprinting or 3D culturing material. The kit contains two components, sterile GelMA powder and chosen photoinitiator (PI) e.g. LAP or Irgacure 2959.

Material needed

- GelMA powder (500 mg), sterile *
- LAP or Irgacure 2959 (100 mg)
- Sterile PBS 1X
- Optional: HEPES buffer (1 M), NaOH or HCl
- Sterile 15 mL Falcon Tube
- Sterile 12 mL syringes, 2 pcs
- Sterile 0.22 µm filter
- Sterile serological pipettes
- Sterile stir bar
- 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. Remove the GelMA powder from storage to reach room temperature.

Step	Title	Material	Description
1	Prepare PBS and PI	- Sterile PBS - Optional: HEPES buffer (1 M)	- Prepare 12 mL, or as much solution as is needed based on Table 1, of PBS or your desired reconstitution solution.

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		- PI of choice - Sterile 12 mL syringe - Sterile 15 mL Falcon tube - Sterile 0.22 µm filter	Note: Examples of reconstitution solutions 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 2. - 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	- Vial of GelMA - Reconstitution solution - Sterile serological pipette - Sterile stir bar - NaOH or HCl - Sterile 12 mL syringes	- 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 1. - Add a sterile stir bar to the vial. - Stir the mixture for 30 minutes at 70°C to ensure dissolution. - 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. 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. - Transfer GelMA solution to the 12 mL syringe and cover with foil to protect from light.
3	Bioprinting or casting	- Female/female luer lock connectors - UV shielding cartridge, 3cc - Well plate	- If using GelMA for bioprinting, attach sterile luer lock to syringe and transfer the GelMA solution to the amber printing cartridge. See *Bioprinting Protocol GelMA* for further details of printing and crosslinking 10% GelMA with 0.25% LAP. For other GelMA concentrations, adjust printing conditions depending on the chosen concentration.

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			- If using GelMA as a 3D culturing material, pipette the material into a well plate or desired mold with or without cells.

Table 1. Suggestions of GelMA concentrations for mixing one vial of 500 mg of GelMA

Desired concentration of GelMA solution	Volume reconstitution solution needed
5% (50 mg/mL)	10 mL
10% (100 mg/mL)	5 mL
15% (150 mg/mL)	3.33 mL

Table 2. Suggestions of PI concentrations for GelMA solution.

PI concentration in GelMA 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

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