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Reconstitution Protocol

GelMA-HAMA 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 HAMA powers. This protocol is intended for the generation of GelMA-HAMA bioinks for 3D bioprinting and 3D cell culturing. The kit contains three components, sterile GelMA powder, sterile HAMA powder and chosen photoinitiator (PI) e.g. LAP or Irgacure 2959. The instructions will direct the reconstitution of a GelMA solution and a HAMA 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, the PI within each component will be the same.

Material needed

- GelMA powder (500 mg), sterile *
- HAMA powder (100 mg), sterile*
- LAP or Irgacure 2959 (100 mg)
- 24 mL sterile PBS 1X
- Optional: HEPES buffer (1 M), NaOH or HCl
- Sterile 12 mL syringes, 4 pcs
- Sterile 0.22 µm filter, 2 pcs
- Sterile stir bars, 2 pcs
- Sterile serological pipettes, 2 pcs
- Sterile 15 mL Falcon Tubes, 2 pcs
- Female/female luer lock connectors*
- UV shielding cartridge, 3cc*

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

Protocol

The HAMA 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 HAMA. Remove the GelMA and HAMA powders from storage to reach room temperature.

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

GelMA-HAMA Bioink	wt% GelMA precursor solution	wt% HAMA precursor solution
5%-1%	10% GelMA	2%
7.5%-1%	15% GelMA	2%
10%-1%	20% GelMA	2%
5%-2%	10% GelMA	4%
7.5%-2%	15% GelMA	4%
10%-2%	20% GelMA	4%

HAMA Precursor Solution Reconstitution Protocol

Step	Title	Material	Description
1	Prepare PBS and PI	- Sterile PBS - Optional: HEPES buffer (1 M) - PI of choice - Sterile 12 mL syringe - Sterile 15 mL Falcon tube - Sterile 0.22 µm filter	- Prepare 12 mL of PBS or your desired reconstitution solution. Note: Examples of reconstitution solution 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 to maintain 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 reconstitution solution using a syringe and 0.22 µm sterile filter into a sterile 15 mL Falcon tube.
2	Prepare HAMA solution	- Vial of HAMA - Reconstitution solution - Sterile serological pipette - Sterile stir bar - Sterile 12 mL syringe	- Using a sterile serological pipette, add the desired volume of the sterilized PI reconstitution solution to the vial of HAMA powder to achieve the desired concentration, see Table 3. - Add a sterile stir bar to the vial. - Stir the solution 30 minutes at room temperature to ensure dissolution. - Transfer HAMA precursor solution to a sterile syringe and cover with foil to protect from light.

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Table 2. Suggestions of PI concentrations for HAMA solution.

PI concentration in HAMA 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 3. Suggestions of HAMA precursor solution concentrations for mixing one vial of 100 mg of HAMA.

Desired concentration of HAMA 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
4% (40 mg/mL)	2.5 mL

GelMA Precursor Solution Reconstitution Protocol

Step	Title	Material	Description
1	Prepare PBS and PI	- Sterile PBS - Optional: HEPES buffer (1 M) - PI of choice - Sterile 12 mL syringe - Sterile 15 mL Falcon tube - Sterile 0.22 µm filter	- Prepare 12 mL of PBS or your desired reconstitution solution. Note: Examples of reconstitution solution 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 achieving 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 4. - 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	- 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 5. - Add a sterile stir bar to the vial. - Stir the mixture for 30 minutes at 70°C to ensure dissolution.

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		- Sterile 12 mL syringe	- Transfer GelMA precursor solution to the 12 mL syringe and cover with foil to protect from light.

Table 4. Suggestions of PI concentrations for 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 5. 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

Mixing GelMA-HAMA Protocol

Step	Title	Material	Description
1	Mix GelMA and HAMA	- Sterile syringe - Luer lock connectors - UV shielding cartridge, 3cc - NaOH or HCl - UV shielding cartridge, 3cc	- Warm up both the GelMA and HAMA 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 HAMA 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. Note: Adding additional liquids to adjust the pH will dilute your bioink and PI concentration. - Transfer the whole volume to one syringe and cap. - Lightly centrifuge (500 rpm) to remove air bubbles - Transfer into 3cc cartridge for bioprinting.

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