Ref. No.: 0070060041.BLA
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GelMA-HAMA Kit Protocol

Kit Components

Item	Quantity	Storage
CELLINK GelMA Powder - Sterile	500 mg	-20 °C, protected from light
CELLINK HAMA Powder - Sterile	100 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
15 mL Falcon Tube - Sterile	1
50 mL Falcon Tube - Sterile	1
Sterile 0.22 µm filter	2
Irgacure 2959 or LAP	200 mg
Sterile Serological pipets	2

Protocol Summary

This kit and protocol is intended for the generation of GelMA-HAMA bioinks for 3D bioprinting. The kit contains two components, sterile GelMA powder and sterile HAMA powder. 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.

Examples of common Compositions and Recipes

GelMA-HAMA Bioink	GelMA wt% Needed	HAMA wt% Needed
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%

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HAMA Precursor Solution Reconstitution Protocol

This HAMA precursor solution will be made at twice the desired final concentration of HAMA, since it will be mixed with the GelMA Precursor solution.

1. Prepare 10 mL of PBS or your desired reconstitution buffer.
2. Mix in the desired amount of photoinitiator to achieve the necessary precursor solution concentration.

Final PI Concentration in Bioink	Mass of PI for 10 ml of Reconstitution Buffer Stock
0.05% (0.5 mg/mL)	5 mg
0.10% (1 mg/mL)	10 mg
0.25% (2.5 mg/mL)	25 mg

3. Sterile filter the photoinitiator solution using the 12 mL syringe and 0.22 µm sterile filter into a sterile falcon tube.

4. Pipet the desired volume of the sterilized reconstitution solution to the vial of CELLINK HAMA powder to achieve the desired concentration.

Final Concentration Desired	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

5. Add a sterile stir bar to the vial.
6. Stir the solution 30 minutes at room temperature to ensure dissolution.
7. Transfer HAMA precursor solution to a syringe.

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GelMA Precursor Solution Reconstitution Protocol

This GelMA precursor solution will be reconstituted at twice the desired final concentration for GelMA concentration since it will be mixed with the HAMA Precursor solution at a ration 1:1.

Remove CELLINK GelMA powder from storage and return to room temperature.

1. Prepare 25 mL of warmed PBS.
2. Mix in the desired amount of photoinitiator to achieve the necessary precursor solution concentration.

Final PI Concentration	PI mass for 25 ml of Buffer Stock
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

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

Final Concentration Desired	Volume Reconstitution Solution Needed
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

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

Mixing GelMA-HAMA

1. Warm up both the GelMA and HAMA precursor solution 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.

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