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Preparation Protocol

GelMA HIGH

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

Protocol aim

The aim of this protocol is to provide instructions for diluting GelMA HIGH into a 10% or 5% bioink with desired concentration of photoinitiator (PI). This protocol is intended for the generation of GelMA bioinks for 3D bioprinting. The GelMA HIGH can also be used for 3D cell culture and casting applications. The instructions will direct the preparation of a GelMA solution and a diluting buffer that is then mixed to generate the bioink.

Material needed

- GelMA HIGH syringe (3 ml), sterile *
- LAP or Irgacure 2959
- Sterile PBS 1X
- Sterile 12-20 mL syringes, 4 pcs
- Sterile 0.22 µm filter
- 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 GelMA HIGH is provided at 20% w/w in PBS at PH of 7.4. This protocol assumes mixing at 1:1 or 1:3 ratio with the diluting buffer to achieve a 10% or 5% GelMA solution. If different final concentrations of GelMA are desired, ensure that the photoinitiator in the diluting buffer is at the right concentration to achieve the desired final concentration required for photocrosslinking. See Table 1 and 2 below for suggestion of compositions to mix up the GelMA HIGH.

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Table 1. Suggestions of compositions of GelMA HIGH for mixing 1:1 GelMA HIGH: Dilution buffer.

Final PI Concentration in Bioink	Concentration in Diluting Buffer	Mass of PI for 5 ml of Diluting Buffer
0.05% (0.5 mg/mL)	0.10% (1.0 mg/mL)	5 mg
0.10% (1 mg/mL)	0.2% (2.0 mg/mL)	10 mg
0.25% (2.5 mg/mL)	0.5% (5.0 mg/mL)	25 mg

Table 2. Suggestions of compositions of GelMA HIGH for mixing 1:3 GelMA HIGH: Dilution buffer.

Final PI Concentration in Bioink	Concentration in Diluting Buffer	Mass of PI for 10 ml of Diluting Buffer
0.05% (0.5 mg/mL)	0.066% (0.66 mg/mL)	6.6 mg
0.10% (1 mg/mL)	0.133% (1.33 mg/mL)	13.3 mg
0.25% (2.5 mg/mL)	0.333% (3.33 mg/mL)	33 mg

HAMA Precursor Solution Reconstitution Protocol

Step	Title	Material	Description
1	Prepare PBS and PI	- Sterile PBS - PI of choice - Sterile 12 mL syringe - Sterile 0.22 µm filter - Sterile 15 mL Falcon tube	- Prepare PBS or your desired diluting buffer. - Mix in the desired amount of PI to achieve the diluting buffer concentration derided, see Table 1 and 2. - Sterile filter the PI solution using a 12 mL syringe and 0.22 µm sterile filter into a sterile 15 mL Falcon tube.
2	Mix GelMA HIGH with diluting buffer	- GelMA HIGH - Sterile syringe - Female/female luer lock connectors	- Warm the GelMA HIGH to 37°C to liquify. - Warm the diluting buffer to 37°C. - Transfer the necessary volume of diluting buffer into a syringe. - Connect the two syringes using the female/female luer lock connector and mix back and forth until the mixture is homogeneous. - Transfer the whole volume to one syringe and cap. - Lightly centrifuge (500 rpm) to remove air bubbles.
3	Bioprint	- UV shielding cartridge, 3cc	- Transfer into 3 cc cartridge for bioprinting. - Refer to the Bioprinting protocol GelMA for future steps of printing and crosslinking GelMA 10%.

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