

RECONSTITUTION PROTOCOL

PhotoGel[®] 50% DS

This is a suggested procedure, please adjust it according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions on how to reconstitute PhotoGel[®] 50% DS (lyophilized GelMA) to your desired concentration using Reconstitution Agent P. Addition of a photoinitiator (PI) and use of 365 or 405 nm LED modules ensure stable and controlled photocrosslinking of GelMA constructs for 3D cell culturing. The obtained GelMA hydrogel can be used as a bioink on its own or as a component in other bioink formulations.

Stability: PhotoGel[®] 50% DS reconstituted in Reconstitution Agent P is stable for at least 6 months without a photoinitiator, and 4 months with a photoinitiator, if stored at 4-8°C protected from light.

Materials needed

- PhotoGel[®] 50% DS (500 mg)*
- Reconstitution Agent P* or an alternative buffer of choice
- Photoinitiator*
- Syringes
- 0.22 µm sterile syringe filter
- 15 mL Falcon tube or equivalent
- Female/female Luer lock adaptors*
- Amber cartridge, 3cc*

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

Protocol

This protocol describes the reconstitution of 500 mg of PhotoGel® 50% DS to obtain GelMA bioinks of different concentrations. All PhotoGel® 50% DS concentrations are calculated as the weight of PhotoGel® 50% DS per volume of Reconstitution Agent P (w/v).

1. Preparing PhotoGel® 50% DS

MATERIAL

PhotoGel® 50% DS

DESCRIPTION

- Take PhotoGel® 50% DS from storage and let it reach room temperature.

2. Preparing Reconstitution Agent P with PI

MATERIAL

Reconstitution Agent P

PI, for example LAP

Syringe

0.22 µm sterile syringe filter

15 mL Falcon tube

DESCRIPTION

- Prepare 12 mL of a Reconstitution Agent P.

Note: Reconstitution Agent P is a specially designed buffer that maintains a physiologic pH in the final GelMA bioink.

- Mix the desired amount of PI in the Reconstitution Agent P. Remember to protect all PI containing mixtures from light. Ensure PI is properly dissolved before proceeding.

Note: LAP is used as the recommended PI for photocrosslinking at 405 nm and is used in the calculations below (see Table 1 for suggested LAP concentrations). For other PI options, amounts may vary.

- Warm the PI solution to ~50°C. Sterile filter the PI solution into a sterile 15 mL Falcon tube using a syringe and 0.22 µm sterile syringe filter. Keep the solution at ~50°C.

Table 1. Suggestions of LAP concentrations for GelMA bioink.

| LAP concentration in GelMA bioink | LAP mass for 12 mL of Reconstitution Agent P |
|-----------------------------------|--|
| 0.10% (1 mg/mL) | 12 mg |
| 0.25% (2.5 mg/mL) | 30 mg |

3. Reconstituting PhotoGel® 50% DS

MATERIAL

PhotoGel® 50% DS

PI solution (Reconstitution agent + PI)

DESCRIPTION

- Add your desired PI solution to the PhotoGel® 50% DS vial, see Table 2 for suggested GelMA bioink concentrations.
- Mix on a shaker table or rotator plate until fully solubilized. Keep warm (37-50°C) if possible (eg. place your rotator in an incubator) to help with full solubilization.
- Double check that the pH is in the 7.0-7.4 range since pH is important for the proper viscosity of the bioink. If needed, balance with small volumes of NaOH or HCl solutions.

Note: Be careful with adding NaOH since high pH degrades GelMA.

- Use the GelMA solution as it is or mix it with other components of choice.
- Warm the GelMA solution at 37°C until it is liquid. Transfer the GelMA solution to syringes and aliquot the volume if applicable.
- Store at 4-8°C.

Table 2. Suggestions of final GelMA bioink concentrations for reconstitution of 500 mg PhotoGel® 50% DS.

| Concentration of GelMA bioink (w/v) | Volume of PI solution needed |
|-------------------------------------|------------------------------|
| 5% | 10 mL |
| 10% | 5 mL |
| 20% | 2 mL |

4. Preparing the GelMA bioink

MATERIAL

Syringe

Female/female Luer lock connectors

Amber cartridge, 3cc

DESCRIPTION

- Warm the needed amount of GelMA solution at 37°C until it is liquid. Transfer the GelMA solution from the syringe to an amber cartridge using a Luer lock connector.
- See the *Bioprinting Protocol GelMA Bioink* for instructions on how to bioprint GelMA 10% with or without cells.