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Reconstitution Protocol

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 HAMA powder for the use in 3D cell culture. The kit contains two components, sterile HAMA powder and chosen photo initiator (PI) e.g. LAP or Irgacure 2959.

Material needed

- HAMA powder (100 mg), sterile*
- 10 mL sterile PBS 1X
- LAP or Irgacure 2959 (50 mg)
- Optional: HEPES buffer (1 M), NaOH or HCl
- Sterile syringes
- Sterile 0.22 µm filter
- Sterile 15 mL Falcon Tube
- Sterile serological pipettes
- Sterile stir bar

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

Protocol

Remove the HAMA powder from storage to reach room temperature.

Step	Title	Material	Description
1	Prepare PBS and PI	- Sterile PBS - Optional: HEPES buffer (1 M) - PI of choice - Sterile 12 mL syringe	- Prepare 12 mL of PBS or your desired reconstitution solution. 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

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		- Sterile 0.22 µm filter - Sterile 15 mL Falcon tube.	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 1. - Sterile filter the PI reconstitution solution using the 12 mL 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 - NaOH or HCl	- Using a sterile serological pipette, add the desired volume of the sterilized reconstitution solution to the vial of HAMA powder to achieve the desired concentration, see Table 2. - Add a sterile stir bar to the vial. - Stir the solution 30 minutes at room temperature 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. - Transfer HAMA precursor solution to a sterile 12 mL syringe. - See the *Casting Protocol HAMA* for casting with cells.

Table 1. Suggestions of PI concentrations for HAMA solution.

PI concentration in HAMA 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 2. Suggestions of final HAMA concentrations for mixing one vial of 100 mg HAMA.

Desired concentration of HAMA 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