

Reconstitution Protocol

ColMA Lyophilizate

This is a suggested procedure, please adjust 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 for reconstituting the ColMA Lyophilizate (collagen methacrylate) into solution. **Please note**, the reconstituted ColMA solution should be further neutralized and supplemented with a photoinitiator prior to mixing with cells and 3D culturing. Refer to the *Neutralization and Printing Protocol ColMA Solution* for the suggested next steps after product reconstitution.

Materials needed

- ColMA Lyophilizate (100 mg), sterile*
- Ice bath
- Reconstitution Agent A* or an alternative sterile acidic solution
- Vortex mixer, shaking table or sterile stir bar (optional)

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

Protocol

This protocol describes reconstitution of 100 mg of ColMA to obtain bioinks of different concentrations.

Step	Title	Material	Description
1	Make calculations	- Calculator (optional)	- Record the desired final ColMA concentration (C_F). See <i>Figure 1</i> for the stiffness of photocrosslinked ColMA, with different C_F . - Calculate the target concentration of the ColMA stock solution (C_S) you need to prepare: $C_S = C_F \times 1.25$ Note: C_F and C_S cannot be the same, otherwise the solution would not be neutralized and supplemented with a photoinitiator.
2	Dissolve ColMA	- Bottle of lyophilized ColMA - Reconstitution Agent A - Ice bath - Vortex mixer/shaking table/Sterile stir bar	- Add the desired volume of the Reconstitution Agent A to the ColMA bottle to achieve the target concentration of the stock solution (C_S), see <i>Table 1</i> . - Add sterile stir bar and mix gently over night at 4°C. Avoid rapid stirring which can generate air bubbles. Alternatively, place the bottle in the fridge and turn the bottle over a couple of times every other hour. - After dissolution, store the vial with ColMA stock solution at 4-8°C.
3	Neuralization & printing		- See the <i>Neutralization and Printing Protocol ColMA Solution</i> for how to neutralize and print a 5 mg/ml ColMA solution.

Table 1. Preparation of ColMA stock solution.

Concentration (C_S), mg/mL	Volume of Reconstitution Agent A, mL
5	20
8	12.5
10	10

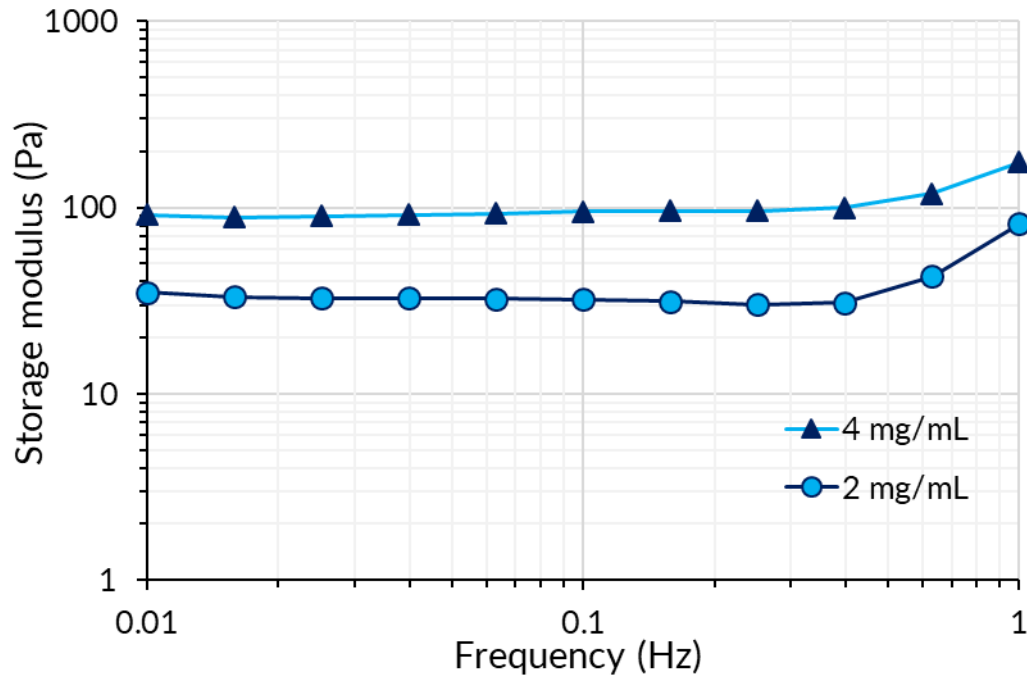


Figure 2. Stiffness of photocrosslinked ColMA biomaterial with different concentration (C_F), containing 0.25% LAP as a photoinitiator. The distance of UV module from the samples was set to 3 cm, and crosslinking time was 30 s.