

ColMA Kit Reconstitution Protocol

Kit Components

Item	Quantity	Storage
CELLINK ColMA Powder - Sterile	100 mg	-20 °C, protected from light

Materials not included

Item	Quantity
Stir Bar – Sterile	1
Sterile 20 mM acetic acid	50 mL
50 mL Tube - Sterile	1
Irgacure 2959 or LAP	200 mg
Sterile 10 X PBS	10 mL
Sterile DI Water	10 mL
Sterile 1 N NaOH	10 mL

Protocol for Casting

1. Record the desired final volume of the ink (V_{INK}).
2. Record the desired final collagen concentration (C_{Fconc}).
3. Add sterile 20 mM acetic acid solution to CELLINK ColMA at the desired volume to achieve the target concentration. Record the concentration of this collagen stock solution (C_{Conc}).

Concentration Desired	Volume Reconstitution Solution Needed
0.3% (3 mg/mL)	33.3 mL
0.5% (5 mg/mL)	20 mL
1% (10 mg/mL)	10 mL

4. Add sterile stir bar and mix gently over night at 4 °C. Avoid rapid stirring which can generate air bubbles.
5. After dissolution, maintain the vial with ColMA stock solution on ice to keep cool.
6. Prepare a neutralization solution for the collagen based on the following calculations:
 - a. Volume of ColMA Stock Solution (V_{ColMA}) = $\frac{C_{Fconc} \times V_{INK}}{C_{Conc}}$
 - b. Volume of 10X PBS (V_{PBS}) = $\frac{V_{INK}}{10}$
 - c. Volume of 1N NaOH (V_{NaOH}) = $V_{ColMA} \times 0.023$
 - d. Volume of DI water (V_{DI}) = $V_{INK} - V_{ColMA} - V_{PBS} - V_{NaOH}$

- e. Mix the volumes from steps b, c and d. This is the neutralization solution (V_{NS})
 - f. (Optional) For photocrosslinking, dissolve the mass of PI in the neutralization solution to achieve a 2.5 mg/mL final concentration. Mass is $V_{INK} \times 0.0025$
 - g. Cool the neutralization solution on ice for 10 minutes.
 - h. Transfer the V_{CoIMA} to the tube containing the V_{NS} , gently mix via pipetting.
7. Cast structure and warm to 37 °C to induce gelation.
 8. If desired and, photoinitiator has been added, crosslink under UV exposure.

Protocol for Bioprinting

1. Record the desired final volume of the ink (V_{INK}).
2. Record the desired final collagen concentration (C_{Fconc}).
3. Add sterile 20 mM acetic acid solution to CELLINK ColMA at the desired volume to achieve the target concentration. Record the concentration of this collagen stock solution (C_{Conc}).

Concentration Desired	Volume Reconstitution Solution Needed
0.6% (6 mg/mL)	33.3 mL
0.8% (8 mg/mL)	20 mL
1% (10 mg/mL)	10 mL
1.2% (12 mg/mL)	8.33 mL
1.4% (14 mg/mL)	7.14 mL
1.6% (16 mg/mL)	6.25 mL

4. Add sterile stir bar and mix gently over night at 4 °C. Avoid rapid stirring which can generate of air bubbles.
5. After dissolution, maintain the ColMA stock solution on ice to keep cool.
6. Prepare a neutralization solution for the collagen based on the following calculations:
 - a. Volume of ColMA Stock Solution (V_{CoIMA}) = $\frac{C_{Fconc} \times V_{INK}}{C_{Conc}}$
 - b. Volume of 10X PBS (V_{PBS}) = $\frac{V_{INK}}{10}$
 - c. Volume of 1N NaOH (V_{NaOH}) = $V_{CoIMA} \times 0.023$
 - d. Volume of DI water (V_{DI}) = $V_{INK} - V_{CoIMA} - V_{PBS} - V_{NaOH}$
 - e. Mix the volumes from steps b, c and d. This is the neutralization solution (V_{NS}).
 - f. (Optional) For photocrosslinking dissolve the mass of PI in the neutralization solution for to achieve a 2.5 mg/mL final concentration. Mass is $V_{INK} \times 0.0025$.
 - g. Chill the neutralization solution on ice for 10 minutes.
 - h. Transfer the V_{CoIMA} to the tube containing the V_{NS} , gently mix via pipetting.
7. Print structure and warm to 37 °C to induce gelation.
8. If desired and, photoinitiator has been added, crosslink under UV exposure.