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Reconstitution Protocol

Coll I Solution

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

Protocol aim

The aim of this protocol is to provide instructions for neutralizing the Coll I solution.

Material needed

- Coll I solution (20 mL at 5 mg/mL), sterile*
- Ice bath
- Sterile 10X PBS
- Sterile deionized (DI) water
- Sterile 1 M NaOH
- Sterile 15 mL Falcon tube

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

Protocol

Step	Title	Material	Description
1	Cool Coll I	- Vial of Coll I - Ice bath	- Place the vial of Coll I stock solution on ice to keep cool.
2	Decide final volume and concentration		- Record the desired final volume of the ink (V_{INK}). - Record the desired final collagen concentration (C_{Fconc}). - C_{Conc} = 5 mg/mL - See Table 1 for example calculation.
3	Calculations for neutralization		- Prepare a neutralization solution for the collagen based on the following calculations: $V_{Col\ I} = \frac{C_{Fconc} \times V_{INK}}{C_{Conc}}$

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			Note: C_{FConc} and C_{Conc} cannot be the same, otherwise the solution would not be neutralized - Volume of 10X PBS: $V_{PBS} = \frac{V_{INK}}{10.58}$ Note: The 10X PBS can be changed to 10X medium. - Volume of 1M NaOH: $V_{NaOH} = V_{Col\,I} \times 0.0196$ - Volume of DI water: $V_{DI} = V_{INK} - V_{Col\,I} - V_{PBS} - V_{NaOH}$
4	Neutralization	- Sterile 10X PBS - Sterile DI water - Sterile 1 M NaOH - Sterile 15 mL Falcon tube - Ice bath - Vial of Coll I	- Mix following volumes from Step 3; V_{PBS}, V_{NaOH} and V_{DI} in a sterile 15 mL Falcon tube. This is the neutralization solution V_{NS}. $V_{NS} = V_{DI} + V_{PBS} + V_{NaOH}$ - Cool the neutralization solution on ice for 10 minutes. - Transfer the $V_{Coll\,I}$ to the tube containing the neutralization solution. Act fast at this step, once the VNS has been added, the collagen solution will begin to self-assemble. Gently mix by pipetting up and down for 3 min. - Check the pH and if needed adjust with more NaOH to achieve a pH between 7-8.
5	Casting		- Cast structure and warm to 37°C to induce gelation, approximately 10-15 min. - See *Casting Protocol Coll I* for more detailed instruction on casting with cells.

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Table 1. Example of Potential Coll 1 Solutions.

$V_{NS} = V_{PBS} + V_{NaOH} + V_{DI}$

V_{INK}	C_{Fconc}	C_{Cconc}	$V_{Coll\,I}$	V_{NS}	V_{PBS}	V_{NaOH}	V_{DI}
1 mL	1.69 mg/mL	5 mg/mL	358 µL	700 µL	100 µL	7 µL	593 µL
2 mL	1.69 mg/mL	5 mg/mL	716 µL	1.4 mL	200 µL	14 µL	1.186 mL
3 mL	1.69 mg/mL	5 mg/mL	1074 µL	2.1 mL	300 µL	21 µL	1.779 mL