Reconstitution Protocol

Coll 1 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 Coll 1 Lyophilizate to your desired concentration using Reconstitution Agent A. Please note, the reconstituted collagen type I solution should be further neutralized prior to mixing with cells and 3D culturing. Refer to the *Neutralization and Printing Protocol Coll 1 Lyophilizate Solution* for the suggested next steps after product reconstitution.

Materials needed

- Coll 1 Lyophilizate (100 mg)*
- Ice bath
- Reconstitution Agent A* or an alternative sterile acidic solution
- Vortex mixer, shaking table or sterile stirring bar

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

Protocol

This protocol describes reconstitution of 100 mg of Coll 1 Lyophilizate to obtain bioinks of different concentrations.

Step	Title	Material	Description
1	Make calculations	- Calculator (optional)	- Record the desired final collagen concentration (C_F). See Figure 1 for the gelation of collagen with different C_F. - Calculate the target concentration of the 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.
2	Dissolve Coll 1 Lyophilizate	- Bottle of lyophilized Coll 1 - Reconstitution Agent A - Ice bath - Vortex mixer/shaking table/Sterile stirring bar	- Add the desired volume of the Collagen Reconstitution Agent to the Coll 1 Lyophilizate bottle to achieve the target concentration of the stock solution (C_S), see *Table 1*. - Add the 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 it over a couple of times every other hour. - After dissolution, maintain the vial with Coll 1 stock solution in cold.

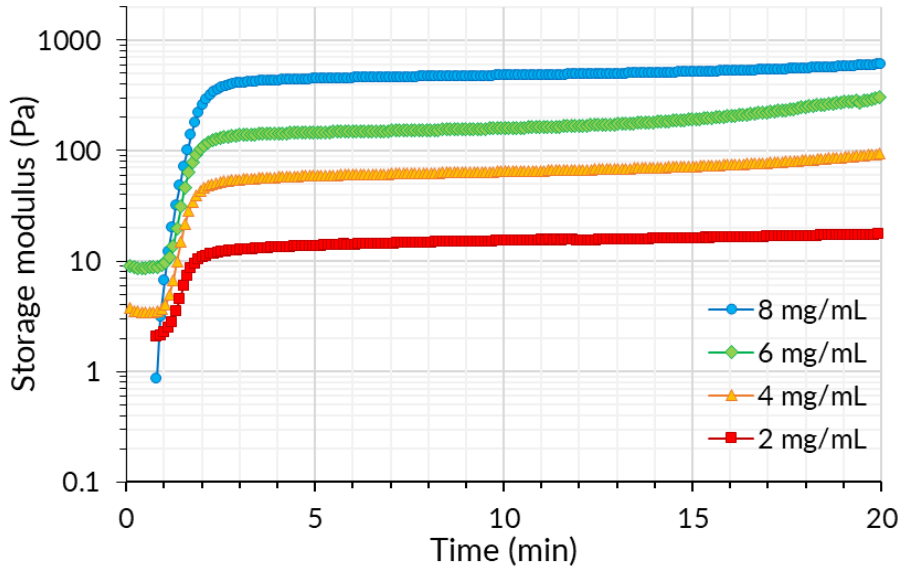

Figure 1. Thermal gelation of neutralized Coll 1 solutions with different collagen concentration (C_F) indicated as storage moduli increase over time at 37°C.

Table 2. Preparation of Coll 1 stock solutions.

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