

## 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 Solution* for the suggested next steps after product reconstitution.

### Materials needed

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

\*The product can be purchased in the CELLINK shop at [www.cellink.com/shop](http://www.cellink.com/shop).

# Protocol

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

## 1. Calculations

### DESCRIPTION

- 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. Dissolving Coll 1 Lyophilizate

### MATERIAL

Coll 1 Lyophilizate

Reconstitution Agent A

Ice bath

Vortex mixer/shaking table/sterile stirring bar

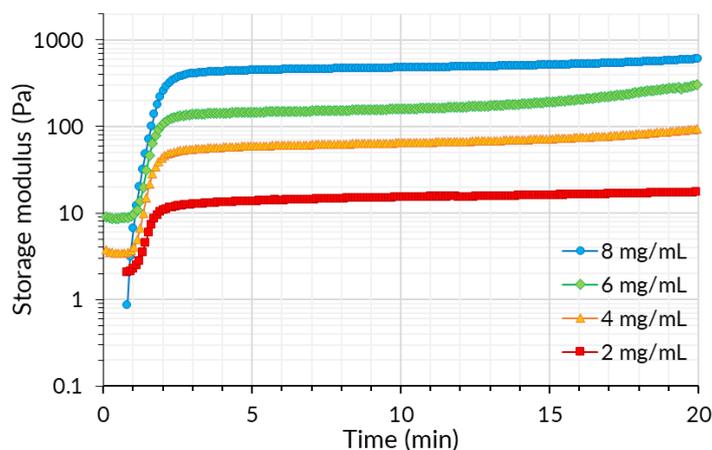
### DESCRIPTION

- Add the desired volume of the Reconstitution Agent A to the Coll 1 Lyophilizate bottle to achieve the target concentration of the stock solution ( $C_S$ ), see Table 1.

**Table 1.** Preparation of Coll 1 stock solutions.

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

- 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 cold.
- See *Printing Protocol Coll 1 Solution* for example on how to bioprint neutralized Coll 1 solution.



**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.