

## DILUTION PROTOCOL

# Alginate 5%

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 on how to dilute the Alginate 5% to your desired concentration using the Reconstitution Agent M. The obtained alginate hydrogel can be used on its own or as an additive in other bioink formulations. The included Crosslinking Agent ensures stable ionic crosslinking of alginate constructs for 3D cell culturing.

### Materials needed

- Alginate 5%\*
- Reconstitution Agent M\* or an alternative buffer of choice
- Syringes with Luer lock connections
- Female/female Luer lock adaptor\*
- Cartridge, 3cc
- Bioprinting nozzles
- BIO X\*, BIO X6\* or INKREDIBLE series\* 3D bioprinter
- Crosslink Agent\* (included with the bioink purchase)

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

# Protocol

All alginate concentrations are calculated as weight of alginate per total weight of Alginate and Reconstitution Agent M (w/w).

## 1. Defining desired concentrations

### DESCRIPTION

- Record the desired final concentration of alginate hydrogel ( $c_F$ ).
- Record the desired final volume of alginate hydrogel to prepare ( $V_F$ ).

See Figure 1 for difference in viscosity of alginate hydrogels at different concentrations.

## 2. Calculations

### DESCRIPTION

- Calculate the volume of Alginate 5% to be used:  $V_{Alg5\%} = \frac{V_F \cdot c_F}{5\%}$   
See Table 1 for suggested  $c_F$ .
- Calculate the volume of Reconstitution Agent M,  $V_R$ , to be used. It is a specially designed buffer that maintains a physiologic pH in the final alginate bioink. It also has a low concentration of ions to prevent premature crosslinking:  $V_R = V_F - V_{Alg5\%}$

**Table 1.** Suggested concentrations and the corresponding volume of Alginate 5% and Reconstitution Agent M used for the preparation of 5 mL alginate hydrogel.

Final concentration of alginate, $c_F$ (%)	Volume of Alginate 5%, $V_{Alg5\%}$ (mL)	Volume of Reconstitution Agent M, $V_R$ (mL)
1	1	4
3	3	2

## 3. Preparing an alginate bioink

### MATERIAL

Reconstitution Agent M

Alginate 5%

Syringes with Luer lock connections

Female/female Luer lock adaptor

### DESCRIPTION

- Transfer  $V_R$  of Reconstitution Agent M into a sterile syringe that can accommodate minimum  $V_F$ .
- Transfer  $V_{Alg5\%}$  of Alginate 5% into another sterile syringe.
- Connect the two syringes using a Luer lock adaptor, make sure there are no air bubbles present. Mix the two solutions by passing them back and forth between the syringes until homogenized.

Note: if air bubbles are introduced into the mixture, centrifuge at  $1000 \times g$  for 1 min to remove them.

Note: If not using the alginate bioink right away, store at 4-8°C.

# 4. Bioprinting

## MATERIAL

Alginate bioink

Cartridge, 3cc

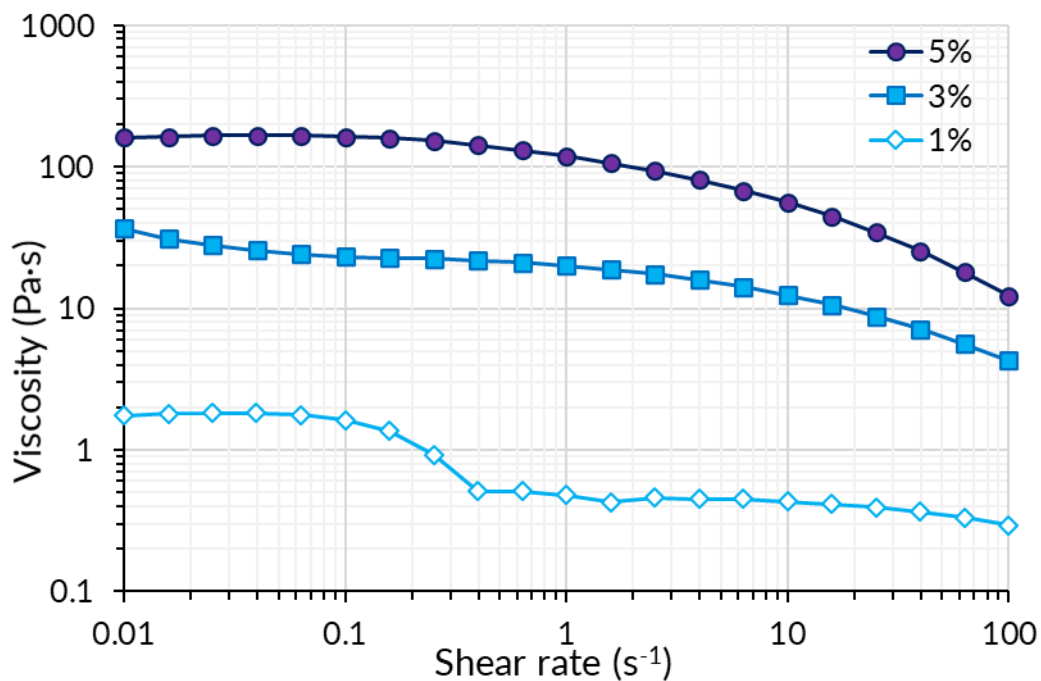
Bioprinting nozzles

BIO X, BIO X6 or INKREDIBLE+

Crosslinking Agent

## DESCRIPTION

- Transfer the alginate bioink to a cartridge and cap with a bioprinting nozzle of choice. See the *Bioprinting Protocol Alginate 5%* for a step-by-step instruction of bioprinting using an alginate bioink.
- Crosslink the bioprinted structures using the Crosslinking Agent for 30 seconds to 5 minutes depending on the size of the constructs.



**Figure 1.** Viscosity of reconstituted alginate at various concentrations over a shear rate range of 0.01 to 100 s<sup>-1</sup>, 25°C.