

## RECONSTITUTION PROTOCOL

# Alginate 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 on how to reconstitute Alginate Lyophilizate to your desired concentration using Reconstitution Agent M. The obtained alginate hydrogel can be used for 3D bioprinting and cell culturing, casting applications and mixing with other biomaterials. Alginate hydrogels can be crosslinked with calcium containing solutions to ensure stable constructs for cell culture.

### Materials needed

- Alginate Lyophilizate (2 x 100 mg), sterile\*
- Reconstitution Agent M\* or an alternative buffer of choice
- Magnetic stir bar
- Cartridges, 3cc
- Bioprinting nozzle
- BIO X\*, BIO X6\* or INKREDIBLE series\* 3D bioprinter
- Crosslinking Agent\*

\*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 Alginate Lyophilizate to obtain bioinks of different concentrations. All alginate concentrations are calculated as weight of alginate per total weight of Alginate and Reconstitution Agent M (w/w).

## 1. Preparing Alginate Lyophilizate

### MATERIAL

Alginate Lyophilizate

### DESCRIPTION

- Take Alginate Lyophilizate from storage and let it reach room temperature.

## 2. Preparing Reconstitution Agent M

### MATERIAL

Reconstitution Agent M

### DESCRIPTION

- Prepare 12 mL of Reconstitution Agent M.

Note: Reconstitution Agent M 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.

## 3. Preparing alginate bioink

### MATERIAL

Alginate Lyophilizate

Reconstitution agent M

Stir bar

### DESCRIPTION

- Add the desired amount of Reconstitution Agent M to the Alginate Lyophilizate vial, see Table 1 for suggested alginate concentrations and Figure 1 for their viscosity.
- Add a sterile stir bar to the container.
- Stir the mixture at room temperature for ~60 minutes, or until dissolved.
- Double check that pH is in the 6.5-7.4 range. If needed, balance with small volumes of NaOH or HCl. If not using right away, store at 4-8°C if needed.

Note: Adding additional liquids to adjust the pH dilutes your bioink.

**Table 1.** Suggestions of final alginate concentrations for reconstitution of 100 mg Alginate.

Concentration of Alginate (w/w)	Volume of Reconstitution Agent M needed
1%	9.9 mL
3%	3.23 mL
5%	1.9 mL

## 4. Bioprinting and crosslinking

### MATERIAL

Alginate bioink

Cartridges, 3cc

Bioprinting nozzle

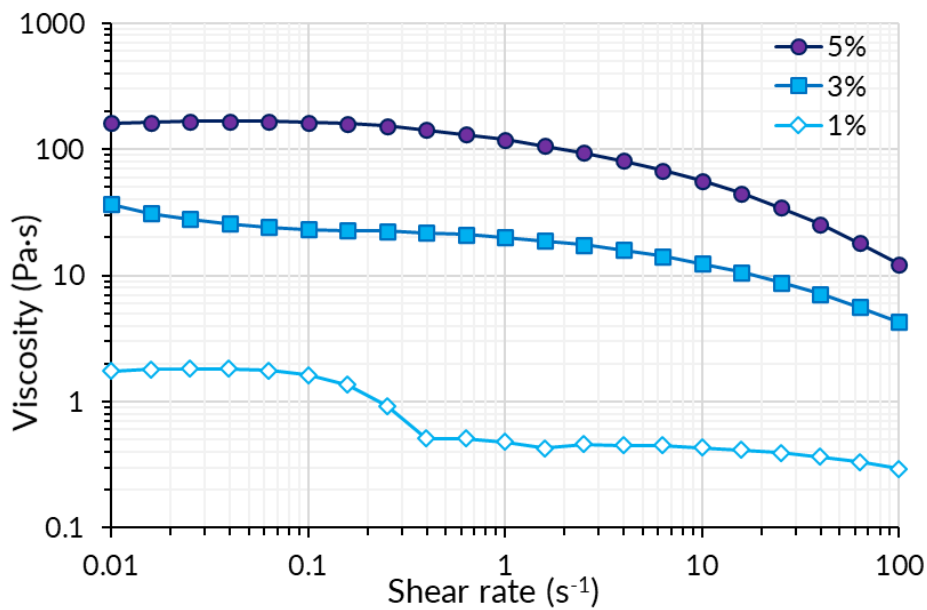
BIO X, BIO X6 or INKREDIBLE bioprinter

Crosslinking Agent

### DESCRIPTION

- Use the alginate bioink as it is or mix it with other components of choice, see *Mixing cells with bioink Protocol* for instructions on how to mix the bioink with cells.
- Transfer the alginate bioink into a cartridge and cap with a bioprinting nozzle of choice.
- Bioprint structures according to application. See the *Bioprinting Protocol Alginate 5%* for an example of printing alginate hydrogel with cells.
- Alginate bioinks can be crosslinked by submerging the bioprinted constructs in Crosslinking Agent for 30 s to 5 min depending on construct size.

Note: 30 seconds is recommended for small droplets while 5 minutes for dense  $>1 \text{ cm}^3$  blocks.



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