

## 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
- Syringes
- Female/female Luer lock adaptors\*
- Crosslinking Agent\*

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

## 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).

Step	Title	Material	Description
1	Prepare Alginate	- Alginate Lyophilizate	- Take Alginate Lyophilizate from storage and let it reach room temperature.
2	Prepare a reconstitution agent	- Reconstitution Agent M	- Prepare 12 mL of a reconstitution agent.  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	Prepare an alginate bioink	- Alginate Lyophilizate - Reconstitution agent M - Stir bar	- Add the desired amount of reconstitution agent 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.  Note: Adding additional liquids to adjust the pH dilutes your bioink.
4	Bioprinting	- BIO X or INKREDIBLE + Alginate bioink - Crosslinking Agent	- Use the alginate bioink as it is or mix it with other components of choice. - Store at 4-8°C. - See the <i>Bioprinting Protocol Alginate 5%</i> for an example of printing alginate hydrogel with cells.

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

Concentration of Alginate (w/w)	Volume of reconstitution agent needed
1%	9.9 mL
3%	3.23 mL
5%	1.9 mL

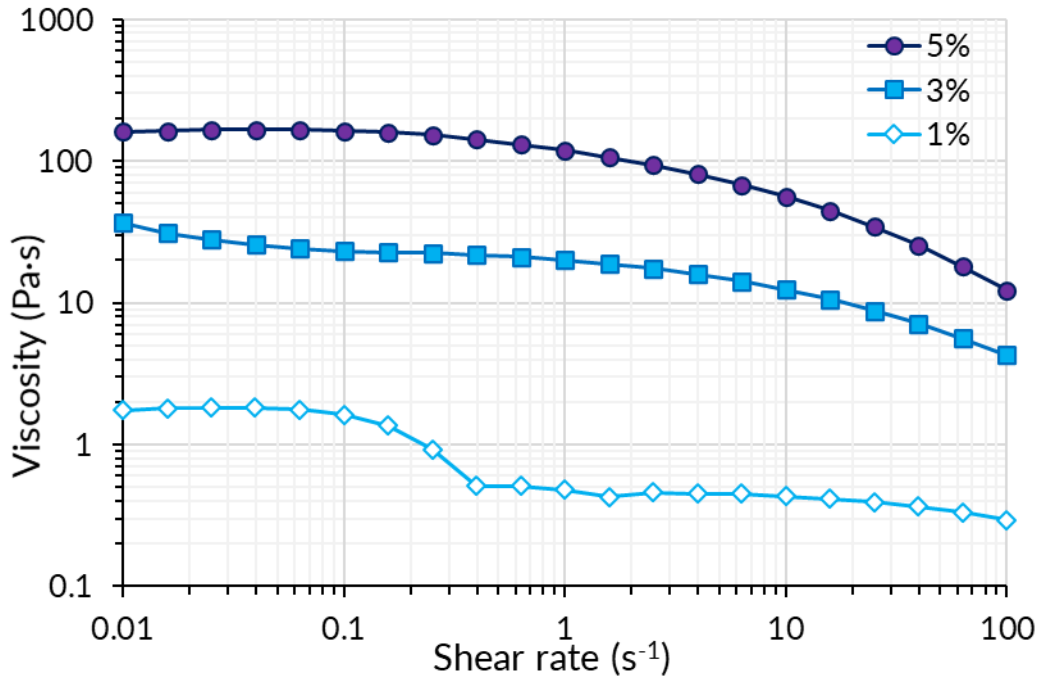


Figure 1. Viscosity of reconstituted Alginate Lyophilizate at various concentrations over a shear rate range of 0.01 to 100 s<sup>-1</sup>, 25°C.