

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 formulation. 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
- Crosslink Agent (CaCl₂)*
- Female/female Luer lock adaptor*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Syringes with Luer lock connections

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

Protocol

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 | Defining desired concentrations | | <ul style="list-style-type: none"> - Record the desired final concentration of Alginate hydrogel (C_F). - Record the desired final volume of Alginate hydrogel to prepare (V_F). <p>See Figure 1 for difference in viscosity of alginate hydrogels at different concentrations.</p> |
| 2 | Calculations | | <ul style="list-style-type: none"> - Calculate the volume of Alginate 5% to be used. $V_{Alg5\%} = \frac{V_F \cdot C_F}{5\%}$ <p>See Table 1 for suggested C_F.</p> <ul style="list-style-type: none"> - 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. $V_R = V_F - V_{Alg5\%}$ |
| 3 | Prepare an alginate bioink | <ul style="list-style-type: none"> - Reconstitution Agent M - Alginate 5% - Syringes - Luer lock adaptors | <ul style="list-style-type: none"> - Transfer V_R of a reconstitution buffer 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. <p>Note: if air bubbles are introduced into the mixture, centrifuge at 1500-2000 rpm for 1-2 min to remove them.</p> |
| 4 | Storage | - Alginate bioink | - Store at 4-8°C. |
| 5 | Bioprinting | <ul style="list-style-type: none"> - BIO X or INKREDIBLE + Alginate bioink | See the <i>Bioprinting Protocol Alginate Solution</i> for a step by step instruction of bioprinting using an alginate bioink. |

Table 1. Suggested concentrations and the corresponding volume of Alginate 5% and reconstitution buffer 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, V_R (mL) |
|--------------------------------------------|------------------------------------------|--------------------------------------------|
| 1 | 1 | 4 |
| 3 | 3 | 2 |

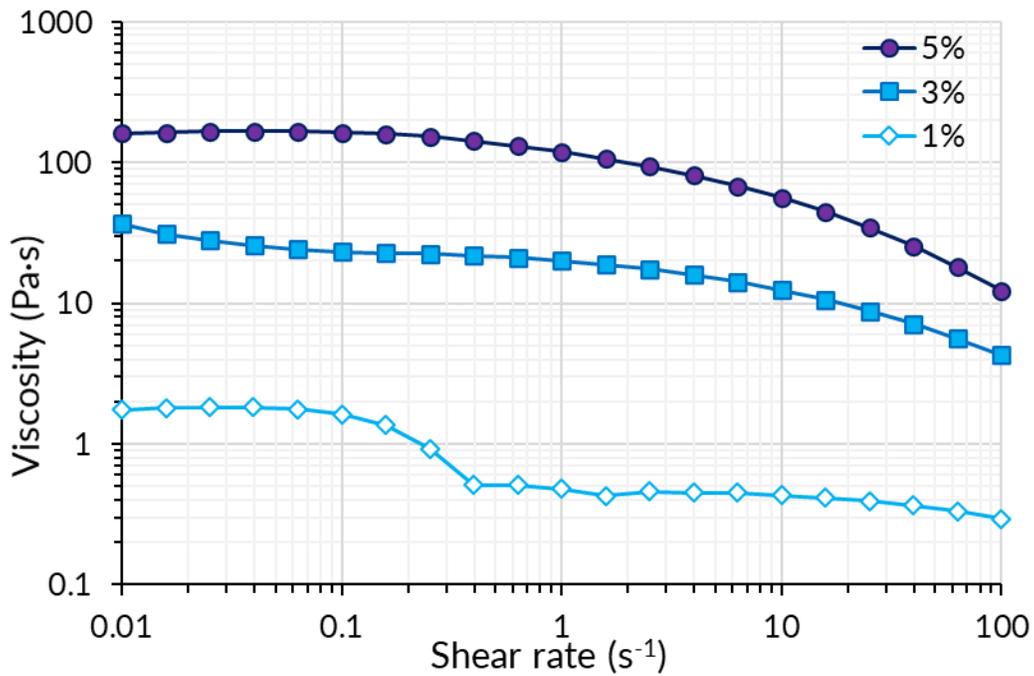


Figure 1. Viscosity of reconstituted alginate at various concentrations over a shear rate range of 0.01 to 100 s^{-1} , 25°C.