

PREPARATION PROTOCOL

CELLINK Xanthan Gum

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 how to use CELLINK Xanthan Gum as a thickener of hydrogels to increase their printability. CELLINK Xanthan Gum is a sterile powder that may be dissolved in water, a buffer solution or a hydrogel. This document includes dissolution of the thickener in a liquid or very low viscosity solution as well as mixing of the dissolved thickener (xanthan gum gel) with a hydrogel for thickening effect.

Materials needed

- CELLINK Xanthan Gum*
- Reconstitution liquid (e.g. water, PBS, HBSS, cell culture medium etc.)
- Tubes
- Hydrogel to be thickened
- Female/female Luer lock adaptor*
- Syringes with Luer lock connections
- Positive displacement pipette + pipette tips (optional)
- Cells* + cell culture medium*
- Cartridges, 3 cc*
- CELLMIXER* (optional)
- BIO X* or INKREDIBLE series* 3D bioprinter
- Bioprinting nozzles* or needles*
- Well plate or Petri dish*

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

Protocol for preparing a xanthan gum gel

1. Calculations

DESCRIPTION

- Record the desired final concentration of xanthan gum (c_x), see Table 1.
- Record the desired volume of gel to prepare (V_L).
- See Figure 1 for difference in viscosity of xanthan gum gels of different concentrations.
- Calculate the amount of xanthan gum to be used: $m_x = 10 \cdot V_L \cdot c_x$

Note: This equation gives a final concentration in weight/volume. However, depending on the concentration of thickener the mixture may swell resulting in a slightly increased final volume.

Table 1. Suggested concentrations and the corresponding amount of xanthan gum powder used for the preparation of 5 mL xanthan gum gel.

Concentration of , c_x (%)	Volume of prepared gel, V_L (mL)	Mass of xanthan gum, m_x (mg)
0.5	5	25
1	5	50
2	5	100
3	5	150

2. Heating up the reconstitution liquid

MATERIAL

Reconstitution liquid

Tube

DESCRIPTION

- Transfer V_L of the reconstitution liquid to a sterile tube or container of your choice.
- To speed up the dissolution of xanthan gum heat up your reconstitution liquid to $\sim 50^\circ\text{C}$ using a water bath, laboratory oven or similar. If your liquid cannot withstand heating, this part can be skipped.

3. Weighing up xanthan gum

MATERIAL

CELLINK Xanthan Gum powder

Tube

DESCRIPTION

- Into a tube, weigh up m_x of xanthan gum powder.

4. Dissolving xanthan gum

MATERIAL

Reconstitution liquid
CELLINK Xanthan Gum

DESCRIPTION

- Into the tube with reconstitution liquid, add the xanthan gum powder. To reduce the formation of clumps, add the xanthan gum in increments and mix with a spatula.
- Vortex the mixture at high speed until dissolved. If clumps form, crush them with a spatula.
- Let the xanthan gum gel rest for a minimum of six hours for it to reach its final viscosity.
- Store at 4-25°C.

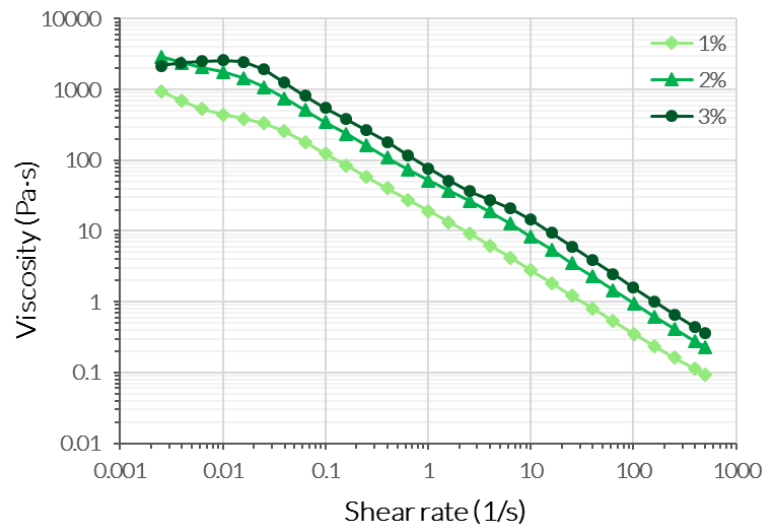


Figure 2. Viscosity of CELLINK Xanthan Gum dissolved in PBS at different concentrations over a shear rate range of 0.002 to 500 s⁻¹, 25°C

Protocol for thickening a hydrogel using xanthan gum

1. Calculating desired final properties

DESCRIPTION

- Record the desired volume of hydrogel and xanthan gum gel mixture (V_2).
- Record the desired final concentration of xanthan gum in the mixture (c_2).
- Record the concentration of xanthan gum gel (c_1). Follow the protocol above to prepare a xanthan gum gel of desired concentration.
- Calculate the volume of xanthan gum gel (V_1) to be used: $V_1 = \frac{V_2 \cdot c_2}{c_1}$
- Calculate the volume of hydrogel (V_H) to be mixed with the xanthan gum gel: $V_H = V_2 - V_1$

2. Mixing xanthan gum gel and hydrogel

MATERIAL

Xanthan gum gel

Hydrogel

Tube

Female/female Luer lock adaptor

Syringes with Luer lock connections

Positive displacement pipette + pipette tips (optional)

DESCRIPTION

- Transfer the calculated volume of xanthan gum gel and hydrogel into a sterile tube or the container of your choice.
- Mix with a sterile spatula.
- Vortex the gel mixture at high speed until it appears homogenous.
- If vortexing is not enough for mixing, use two syringes instead. Transfer the mixture to a syringe, connect the syringe with another syringe of the same size using a female/female Luer lock adaptor. Mix by pushing the gel back and forth between the two syringes. This method may introduce air bubbles that can be removed by centrifuging the syringe for 1-2 min at 300 g.

Note: Transferring viscous gels may be difficult using a normal pipette. If available, use a positive displacement pipette instead.

3. Mixing with cells

MATERIAL

3 mL syringes with Luer lock connections

Female/female Luer lock adaptor

Hydrogel

Cell suspension

Cartridge, 3cc

DESCRIPTION

- At this point, mix ten parts of hydrogel with one part of cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing cells with bioink Protocol*.
- If preparing for quantities < 2 mL of hydrogel, it is recommended to connect two 3 mL Luer lock syringes, one with the bioink and the other with the cell suspension and gently mix back and forth between the syringes until homogeneous. Transfer the mixture to an empty 3cc cartridge by connecting the syringe to the cartridge using the Luer lock adaptor. Cap the cartridge with a tip cap.
- If using larger quantities, the CELLMIXER can be used:
 - Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor.
 - Transfer the hydrogel to the 12 mL syringe (PART 2) using a female/female Luer lock adaptor.
 - Clip both syringes to the Dispensing unit (PART 3).
 - Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing unit's other side.
 - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. Cap the cartridge with a tip cap.

Note: To avoid introducing air when connecting the syringes, carefully pre-fill the Luer lock adaptor with hydrogel before attaching it to the syringe with the cell suspension.

4. Printing

MATERIAL

BIO X, BIO X6 or INKREDIBLE series bioprinter

Bioprinting nozzles or needles

Well plate or Petri dish

DESCRIPTION

- Cap the cartridge with a bioprinting nozzle or a needle. Place the cartridge in the printhead of the 3D bioprinter and connect with the air adapter. Place the well plate or Petri dish on the print bed.
- 3D bioprint the hydrogel mixture according to the application.