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RECONSTITUTION PROTOCOL

Pluronics Powder

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 reconstituting Pluronics Powder. Pluronics is a sterile powder that may be dissolved in water or a buffer solution of your choice. Dissolved Pluronics exhibits high viscosity at room temperature and becomes liquid when cooled.

Materials needed

- Pluronics Powder*
- Reconstitution liquid (e.g. water, PBS, HBSS, cell culture medium etc.)
- Tubes
- Ice bath (for cooling)
- Spatulas/spoons
- Magnetic stirring plate
- Magnetic stir bar
- Cartridges, 3cc*
- BIO X*, BIO X6* or INKREDIBLE series* 3D bioprinter
- Bioprinting nozzles* or needles*

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

Protocol

1. Calculations

DESCRIPTION

- Note the desired final concentration of Pluronics (c_P). To achieve a gel that is 3D printable at room temperature, the recommended final concentration is 40% w/v, c_P.= 40.
- Record the desired volume of gel to prepare, in mL (V_L).
- See Figure 1 for difference in viscosity of Pluronics gels of different concentrations.
- Calculate the amount of Pluronics powder to be used: $m_P = \frac{V_L \cdot c_P}{10}$
- See Table 1 with calculations for suggested c_P.

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

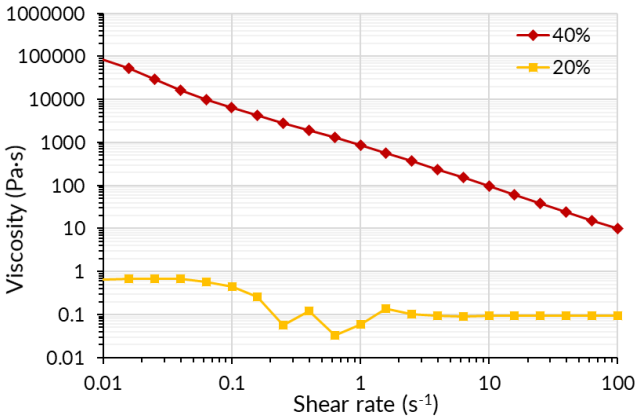

Figure 1. *Viscosity of Pluronics dissolved in deionized water at 20% and 40% over a shear rate range of 0.01 to 100 s^{-1}, 25°C.*

Table 1. Suggested concentrations and the corresponding amount of Pluronics powder used for the preparation of 3 mL Pluronics gel.

Concentration of Pluronics gel, c_P (%)	Volume of reconstitution liquid, V_L (mL)	Mass of Pluronics powder, m_P (g)
20%	3	0.6
40%	3	1.2

2. Preparation of reconstitution solution

MATERIAL

Reconstitution liquid

Tube

DESCRIPTION

- Transfer V_L of the reconstitution liquid to a centrifuge tube or container of choice.
- Cool your reconstitution liquid to ~4°C using an ice bath or fridge.

Note: Since dissolved Pluronics becomes liquid at low temperature, while being a gel at room temperature, the dissolution must be performed at low temperatures when making highly concentrated Pluronics gels. See Figure 2 for temperature dependence of 20% and 40% Pluronics.

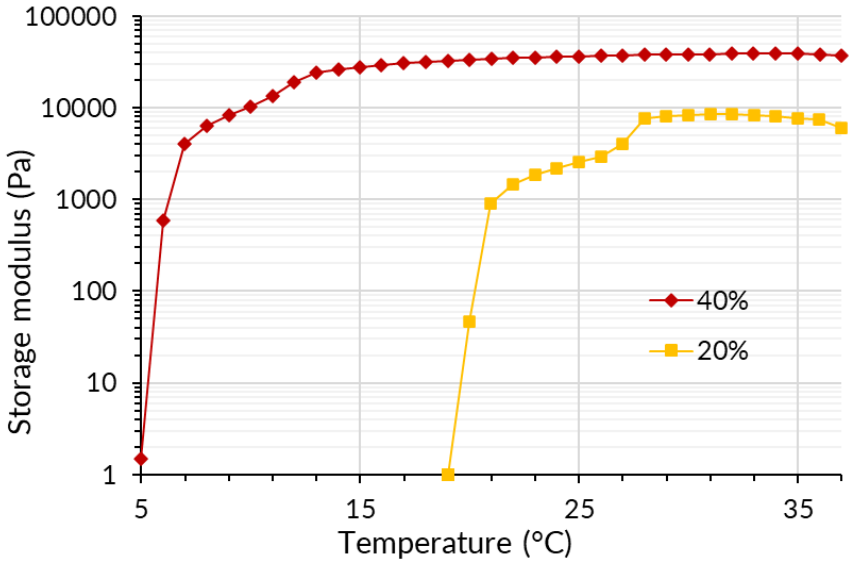

Figure 2. *Storage modulus of Pluronics dissolved in deionized water at 20% and 40% concentration, over a temperature range of 5-37°C.*

3. Preparation of Pluronics

MATERIAL

Spatula/spoon

Tube

Pluronics Powder

DESCRIPTION

- In another tube, weigh up mP of Pluronics Powder using a spatula/spoon.

4. Pluronics dissolution

MATERIAL

Cooled reconstitution liquid

Pluronics Powder

Magnetic stir bar

DESCRIPTION

- Into the tube with reconstitution liquid, add the Pluronics Powder. To reduce the formation of clumps, add the powder in increments and mix with a spatula.
- Add a magnetic stir bar to the container.
- Stir the mixture at high speed in an ice bath until dissolved. If clumps form, crush them with a spatula or shake the container.
- Store the Pluronics gel at 4-8°C.

5. 3D printing

MATERIAL

Pluronics gel

BIO X, BIO X6 or INKREDIBLE series 3D bioprinter

Bioprinting nozzle or needle

DESCRIPTION

- Transfer the Pluronics gel to a cartridge and cap with a bioprinting nozzle or needle.
- 3D print according to application. For suggestions on 3D printing using 40% Pluronics gel as a sacrificial ink, see the *Printing Protocol Pluronics 40%*.