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PRINTING PROTOCOL

# Pluronics 40%

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 3D printing of Pluronics 40% as a sacrificial ink, using the INKREDIBLE Series, the BIO X or BIO X6. This document covers pre-print operations, 3D printing and post-print processes such as removing the sacrificial ink through washing with cold PBS. Diluting Pluronics 40% will reduce its printability.

## Materials needed

- Pluronics 40% *
- Cartridges, 3 cc*
- Female/female Luer lock adaptor*
- Conical bioprinting nozzles, 22-27G*
- BIO X*, BIO X6* or INKREDIBLE-Series* 3D bioprinter
- Petri dish* or well plate
- Cold PBS (maximum 8°C)
- Ice

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

# Protocol

Pluronics 40% has been optimized for the INKREDIBLE-series and BIO X/BIO X6 system equipped with a pneumatic printhead.

## 1. Ink preparation

**MATERIAL**

Pluronics 40%

Cartridge, 3 cc

Female/female Luer lock adaptor

Conical bioprinting nozzles, 22-27G

**DESCRIPTION**

- Connect the Pluronics syringe to a cartridge using a Luer lock adaptor and transfer desired amount into the cartridge.
- Heat Pluronics 40% to room temperature. At temperatures below 13°C it becomes very liquid and thus loses its printability, see Figure 1 for temperature sweep of Pluronics 40%.
- Cap the cartridge with a bioprinting nozzle and place the cartridge in the printhead.

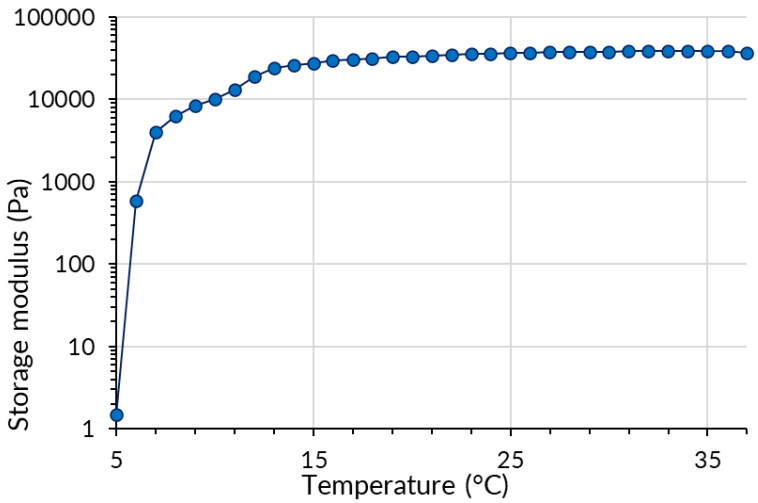


***Figure 1.*** *Storage modulus of Pluronics 40% over a temperature range of 15 to 37°C.*

## 2. Printing

**MATERIAL**

BIO X, BIO X6 or INKREDIBLE series bioprinter

Petri dish or well plate

**DESCRIPTION**

- Print support and/or sacrificial structures using the ink on to a Petri dish or well plate. If printability is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material. The printing pressure is inversely proportional to the nozzle diameter and printing speed.
- The relation between printing pressure and speed for 22–27G nozzles can be seen in Figure 2.

Note: Test the flow of the bioink after the calibration is performed and start with a low pressure and increase stepwise.

Example: If using a 22G nozzle and 10 mm/s printing speed, start at 70 kPa and adjust as needed.

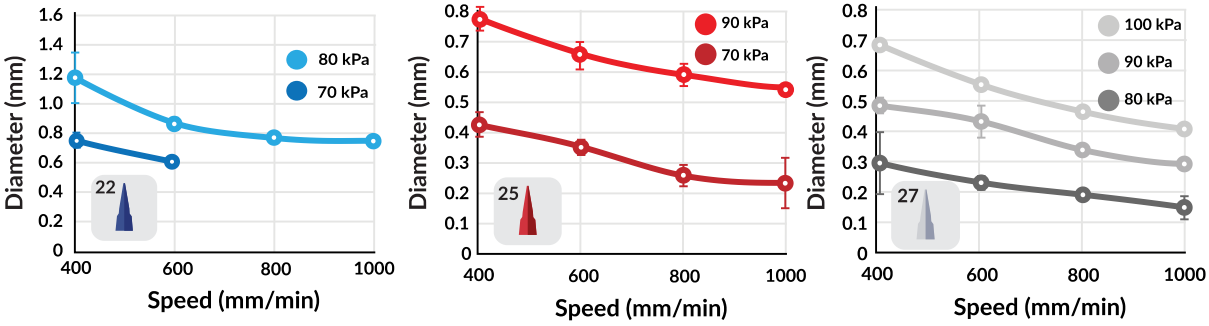


***Figure 2.*** *Filament diameter of Pluronics 40%, 3D printed with various nozzles, pressure, and printing speed, at 25°C.*

# 3. Washing

**MATERIAL**

Cold PBS

Ice

**DESCRIPTION**

- Pluronics 40% becomes liquid at low temperatures and may thus be removed by washing with cold PBS, or your liquid of choice. Prior to washing, make sure to crosslink any other bioinks printed to retain their structure.
- Cover the printed construct in cold PBS and remove by pipetting. Repeat until all Pluronics is removed.
- If the Pluronics is embedded inside a construct, wash with cold PBS and use negative pressure to remove the ink.
- Acellular and large constructs benefit from being placed on ice for faster liquification of the Pluronics.

# 4. Incubation

**MATERIAL**

Cell culture medium

**DESCRIPTION**

- After removing the Pluronics, add the desired medium to the constructs and place in incubator.
- Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% $CO_2$ and 95% relative humidity) or according to your application.