

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

Material needed

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

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

Protocol

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

Step	Title	Material	Description
1	Ink preparation	<ul style="list-style-type: none"> - Pluronic 40% - Cartridge, 3 cc - Female/female Luer lock adaptor - Conical bioprinting nozzles, 22-27G 	<ul style="list-style-type: none"> - Heat Pluronic 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 Pluronic 40%. - Connect the syringe of Pluronic to a cartridge using a Luer lock adaptor. Transfer desired amount into the cartridge and cap with a bioprinting nozzle. Place the cartridge in the printhead.
2	Printing	<ul style="list-style-type: none"> - INKREDIBLE-Series or BIO X 3D bioprinter 	<ul style="list-style-type: none"> - 3D print support and/or sacrificial structures using the ink. 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 a nozzle diameter and printing speed. - The relation between printing pressure and speed for 22–27 G nozzles can be seen in Figure 2. <p>Example: If using a 22 G nozzle and 10 mm/s printing speed, start at 70 kPa and adjust as needed.</p>
3	Washing	<ul style="list-style-type: none"> - Cold PBS - Ice 	<ul style="list-style-type: none"> - Pluronic 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 Pluronic is removed. - If the Pluronic 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 Pluronic.
4	Incubation	<ul style="list-style-type: none"> - Cell culture medium 	<ul style="list-style-type: none"> - After removing the Pluronic, add the desired medium to the constructs and place in incubator. - Incubate the constructs in cell culture medium in standard culture conditions (37°C,

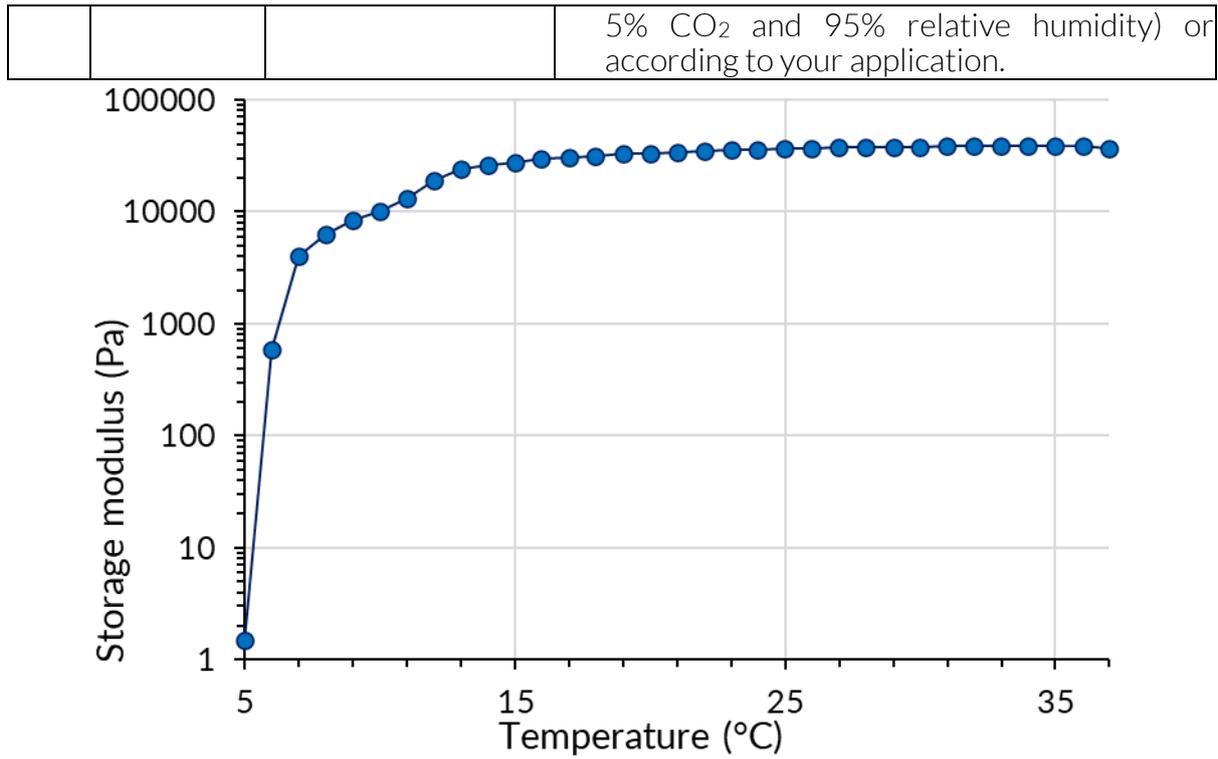


Figure 1. Storage modulus of Pluronic 40% over a temperature range of 15 to 37°C.

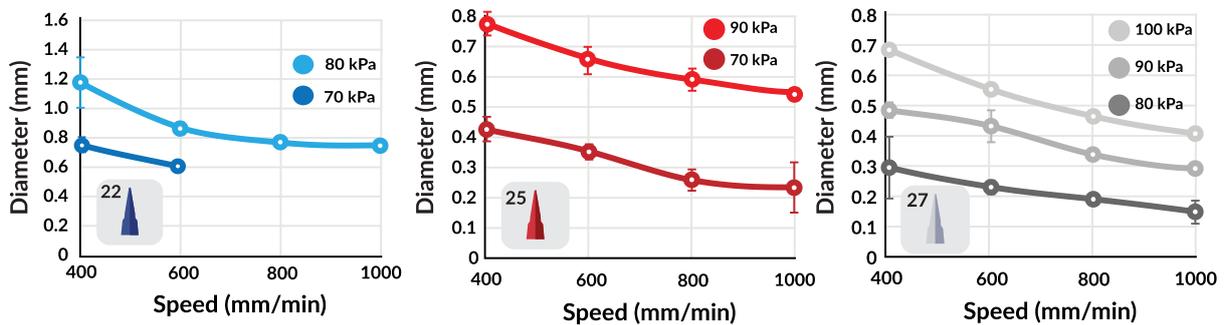


Figure 2. Filament diameter of Pluronic 40%, 3D printed with various nozzles, pressure, and printing speed, at 25°C.