

Bioprinting Protocol

CELLINK Bioink

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 bioprinting with the CELLINK® Bioink using the INKREDIBLE, INKREDIBLE+, BIO X, or BIO X6. It covers steps from pre-print mixing with cells, 3D bioprinting and post-print processes of ionic crosslinking. This protocol was optimized for CELLINK Bioink, undiluted as well as using a 10+1 cell suspension dilution. Changing the parameters in the protocol might change printing pressure or the crosslinking time required. This protocol was optimized using the pneumatic printhead.

Material needed

- CELLINK Bioink*
- Crosslinking Agent*, included with the product
- Clear cartridges, 3cc*
- Sterile Conical Bioprinting nozzles, 22-27G recommended*
- BIO X*, BIO X6* or INKREDIBLE-series* 3D Bioprinter
- Well plate or Petri dish

- Cells + cell culture medium
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
- or
- CELLMIXER*

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

Protocol

This protocol can be performed with printheads and print bed at room temperature, where room temperature is between 20-25°C.

Step	Title	Material	Description
1	Prepare bioink	- CELLINK Bioink	If not printing with cells move directly to step 3. - Warm up CELLINK Bioink in a cartridge to room temperature.
2	Mix CELLINK Bioink with cells	- 3 mL syringes with Luer lock connections - Prewarmed CELLINK Bioink - Female/female Luer lock adaptor - Cell suspension in syringe	At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the <i>Mixing Cells Protocol CELLINK Series</i> . - Transfer the cell suspension to the 1 mL cell syringe (PART 1) using a female/female Luer lock adaptor. - Transfer the bioink 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 units other side. - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with CELLINK Bioink before attaching the syringe with the cell suspension. If preparing for quantities < 2 mL of CELLINK Bioink, it is recommended to connect two 3 mL Luer lock syringes and mix back and forth between the syringes until homogeneous.
3	Cool and load the cartridge	- Clear cartridges, 3cc loaded with CELLINK Bioink (and cells) - Sterile Conical Bioprinting nozzles.	- Place the room tempered CELLINK Bioink in the printhead and cap with a printing nozzle of choice. Note: The recommended nozzle size is 22G. Decreases the nozzle diameter to achieve smaller filament diameter, however this also increase the risk of the bioink clogging.
4	Printing	- Bioprinter (BIO X, BIO X6 or INKREDIBLE series recommended)	- Bioprint structures with parameters according to Table 1, onto a well plate or Petri dish. If printability is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material.

	- Well plate or Petri dish	- Note: If waiting too long between extrusions the bioink can dry in the nozzle causing it to clog. If this occurs, replace with new nozzle.
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Table 1. Recommended minimal extrusion pressure** (± 2 kPa) used for printing continuous filaments at 20-25°C with cells/without cells. Again, 'with cells' assumes a mixture of one part cell suspension to ten parts bioink. For highly concentrated cell suspensions, the pressure needs to be increased towards the pressure used for undiluted bioink.

Printing speed (mm/s) → Nozzle size (G) ↓	5	10	15	20
22	6 / 11	8 / 13	9 / 14	11 / 17
25	8 / 12	10 / 15	11 / 17	12 / 17
27	10 / 13	14 / 15	15 / 17	16 / 20

** This is only a recommended reference of starting pressures. The actual pressure needed will vary depending on the preparation procedures (amount of bioink and actual temperature of the bioink) as well as the fitting of the piston in the cartridge and the leveling of the print surface. This table was generated with printhead temperature of 22°C and with a 10+1 bioink dilution with cell suspension.

Step	Title	Material	Description
5	Crosslinking	- Crosslinking Agent - Cell culture medium	CELLINK Bioink can be crosslinked using the CaCl ₂ -containing Crosslinking Agent. - Submerge the cell-laden constructs in the crosslinking solution for 30 seconds to 5 minutes depending on construct size. Remove crosslinking solution and rinse constructs with basal culture media once.
6	Incubation	- Cell culture medium	- After crosslinking and washing, 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 application.