

BIOPRINTING PROTOCOL

CELLINK A-RGD

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

Materials needed

- CELLINK A-RGD*
- Cells* + cell culture medium*
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
- CELLMIXER* (optional)
- Green or clear cartridges, 3cc*
- Conical bioprinting nozzles*
- BIO X*, BIO X6* or INKREDIBLE-series* 3D bioprinter
- Well plate or Petri dish*
- Crosslinking Agent (included with the bioink purchase)

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

Protocol

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

1. Preparing the bioink

MATERIAL

CELLINK A-RGD

DESCRIPTION

- Warm up CELLINK A-RGD in a cartridge to room temperature.
- *If not printing with cells, move directly to step 3.*

2. Mixing the bioink with cells

MATERIAL

3 mL syringes with Luer lock connections

Female/female Luer lock adaptor

Pre-warmed CELLINK A-RGD

Cell suspension

Cartridge, 3cc

CELLMIXER (optional)

DESCRIPTION

- 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 *Mixing cells with bioink Protocol*.
- If preparing for quantities < 2 mL of CELLINK A-RGD, 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 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 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 CELLINK A-RGD before attaching it to the syringe with the cell suspension.

3. Preparation for printing

MATERIAL

CELLINK A-RGD mixed with cells (if applicable) in cartridge
Conical bioprinting nozzles

DESCRIPTION

- Cap the room tempered CELLINK A-RGD with a bioprinting nozzle of choice and place in the printhead. Connect the cartridge to the air adapter.

Note: Be careful not to touch the printhead with the nozzle tip and if using very liquid materials, make sure that the bioink does not drip through the nozzle especially when attaching the air adapter. Alternatively, the cartridge can be placed in the printhead with the tip cap on and when in place switched to a nozzle.

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

4. Printing

MATERIAL

BIO X, BIO X6 or INKREDIBLE series bioprinter
Well plate or Petri dish

DESCRIPTION

- Bioprint structures using suggested parameters (Table 1) on to 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.

Note: For printing complicated structures, it is recommended that CELLINK A-RGD is used in conjugation with CELLINK START to aid in its stability or with the use of the Syringe Pump printhead to better control extrusion rates.

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	3 / 4	3 / 4	4 / 4	4 / 5
25	4 / 4	4 / 5	5 / 6	6 / 7
27	5 / 6	6 / 8	7 / 9	10 / 11

**Note 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 bioink temperature of 24°C and with a 10+1 bioink dilution with cell suspension.

5. Crosslinking

MATERIAL

Crosslinking Agent
Cell culture medium

DESCRIPTION

CELLINK A-RGD can be crosslinked with ions using the CaCl₂-containing Crosslinking Agent.

- Submerge the cell-laden constructs in the Crosslinking Agent for 30 seconds to 5 minutes depending on construct size, infill density and desired construct stiffness. Remove Crosslinking Agent and rinse constructs with basal culture media once.

Note: 30 seconds is recommended for 10 µL droplets while 10 minutes might be required for dense 1 cm³ blocks. In addition, optimize the crosslinking depending on the cell type.

6. Incubation

MATERIAL

Cell culture medium

DESCRIPTION

- After crosslinking and washing, add the desired medium to the construct and place in incubator.
- Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% CO₂ and 95% relative humidity) or according to your application. Replace medium regularly.