

BIO ONE BIOPRINTING PROTOCOL

CELLINK START

This is a suggested procedure, please adjust it according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for dispensing droplets and bioprinting multi-layered grids with CELLINK START (non-sterile) using the BIO ONE bioprinter. Droplets and grids printing demonstrate the versatility of CELLINK START for simple and complex structures used in endless applications for training purposes. This document covers procedures for printing without cells on Petri dishes or in 96-well plates including suggested bioprinting parameters.

Materials

- CELLINK START*
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Conical bioprinting nozzles, 22-27G recommended*
- BIO ONE 3D bioprinter*
- Well plate or Petri dish*

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

Protocol

The protocol has been optimized for use with the BIO ONE bioprinter. This protocol can be performed with printhead and print bed at room temperature, where room temperature is between 20-25°C.

1. Preparation for printing

MATERIAL

CELLINK START

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

Conical bioprinting nozzles, 22-27G recommended.

DESCRIPTION

- Transfer CELLINK START to the 3 mL syringe using the Luer lock adapter and cap the syringe with a printing nozzle of choice, 22-27G recommended.
- Place the syringe into the printhead. Rotate the syringe plunger holder arm over the plunger and twist the syringe by the tabs counterclockwise to ensure it is locked in place.

2. Calibration and nozzle priming

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Place a Petri dish or well plate on the printbed and perform either manual- or automatic calibration. Perform calibration each time a new syringe is placed in the printhead. If needed, perform Autobed levelling.
- Right before each print, prime the nozzle by extruding a couple of drops of material. Make sure the nozzle tip is clean before starting the print.

Notes:

- Before starting the print, test the flow of the bioink using the Test extrude button with the recommended starting parameters in Table 1 or even lower extrusion rate to avoid losing material during the nozzle priming procedure.
- If the system has been idle for an extended period, the bioink in the nozzle can dry causing it to clog. If this occurs purge the nozzle by extruding 10 to 50 µL of the bioink or until the dried part is extruded. If the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with bioink prior to printing.

3. Printing

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Dispense droplets or print three-layered grids with parameters according to Table 1 or Table 2 respectively, in a well plate or Petri dish.
- See Figure 1 for reference droplets and Figure 2 for three-layered grid structures.
- If the printed structures are not as desired, adjust the extrusion rate up/down by 0.1 μL to extrude more/less material or refer to the BIO ONE Protocol Parameter Guidelines.

Notes:

- The values are only a reference of starting parameters. The actual values needed to print will vary depending on the preparation procedures well as the print surface.

Table 1. Recommended settings in DNA Studio Core used for dispensing 5 μL droplets of CELLINK START through a 22G nozzle in a 96-well plate using the Droplet Print function on the BIO ONE bioprinter.

Parameters	
Well plate	96-well plate
Printbed temperature	-
Printhead temperature	-
Extrusion rate	60 $\mu\text{L/s}$
Extrusion volume	20 μL
Retract volume	15 μL
Z-offset	0.3 mm
Extra preflow volume	0 μL
Retract rate	60 $\mu\text{L/s}$
Postflow stop time	0.3 s

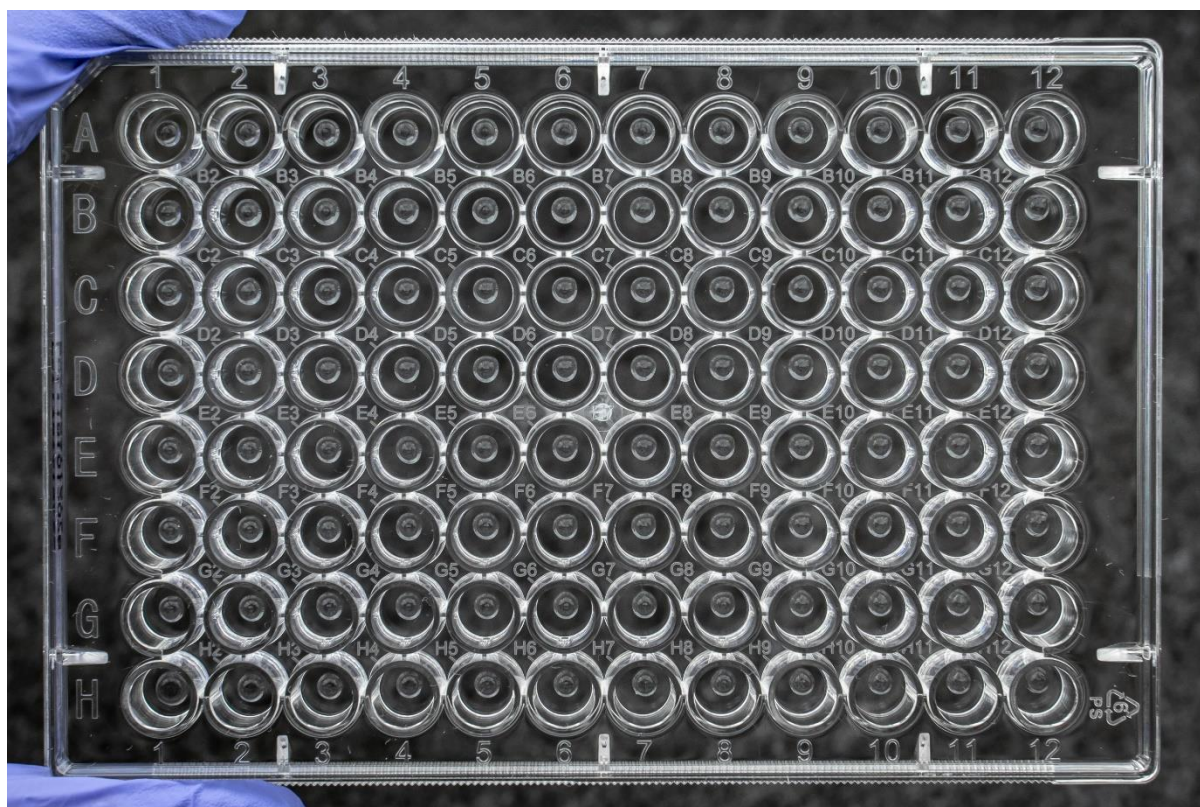


Figure 1. 5 μL droplets of CELLINK START dispensed in a 96-well plate, using parameters in Table 1.

Table 2. Recommended settings in DNA Studio Core used for printing three-layered grids (20 x 20 mm) of CELLINK START.

Parameters	
Surface	Petri dish
Printbed temperature	-

Nozzle	0.2 mm (27G)
Speed	10 mm/s
Printhead temperature	-
Preflow volume	38 μ L
Extrusion rate	2.5 μ L/s
Retract volume	38 μ L
Z-offset	0.1 mm
Extra preflow volume	12 μ L
Infill extrusion multiplier	100%
Retract rate	60 μ L/s
Extra retract	0 μ L
Postflow stop time	0.3 s
Z-lift	3.0 mm



Figure 2. Three-layered grid structure, 20 x 20 mm, acquired after printing with the parameters in Table 2 with CELLINK START.

4. Removal of CELLINK START

MATERIAL

PBS or liquid of choice

DESCRIPTION

- CELLINK START cannot be crosslinked. If CELLINK START has been used as a sacrificial material together with another bioink, make sure that the other bioink is properly crosslinked before adding PBS (or liquid of choice) to flush away the sacrificial CELLINK START from the other bioink.