

Corning® Matribot® Bioprinter: Bioprinting with Corning Pluronics Sacrificial Ink

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Protocol

This is a suggested procedure, please use this as a starting point and adjust according to your experimental needs. To maintain the sterility of the product, work under aseptic conditions.

Introduction

The aim of this protocol is to provide instructions for dispensing droplets and bioprinting multi-layered grids with Corning Pluronics sacrificial ink using the Corning Matribot bioprinter. Droplet dispensing and grid printing reveals the versatility of Corning Pluronics sacrificial ink for simple and complex structures used in several applications for sacrificial purposes. This document covers bioprinting parameters and procedures for dispensing into 96-well microplates and printing onto Petri dishes without cells.

NOTE: Avoid mixing this ink with cells. Corning Pluronics sacrificial ink is intended for use as a sacrificial ink. It can be used with other bioinks to create a scaffold and should be washed away using cold PBS prior to the addition of cells.

For more details on operating the Corning Matribot bioprinter, please refer to the Corning Matribot Bioprinter Instruction Manual (CLS-AN-641DOC).

Materials

- ▶ Corning Matribot bioprinter (Corning 6150)
- ▶ Corning Pluronics sacrificial ink, 2.7 mL/syringe (Corning 6157)
- ▶ 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- ▶ Cold PBS (maximum temperature 8°C)
- ▶ Cell culture medium
- ▶ Petri dish, multiwell plate, or microplate
- ▶ Corning standard or high precision conical bioprinting nozzles, 22-27G*
 - Standard nozzles: 22G-410 μm (Corning 6167); 25G-250 μm (Corning 6166); 27G-200 μm (Corning 6165)
 - High precision nozzles: 22G-410 μm (Corning 6170); 25G-250 μm (Corning 6169); 27G-200 μm (Corning 6168)

*It is recommended to use high precision conical bioprinting nozzles for applications where having low variation in droplet size of samples is crucial.

Protocol

The protocol has been optimized for use with the Corning Matribot bioprinter.

Step	Title	Material	Description
1	Ink preparation	<ul style="list-style-type: none">• 3 mL syringe of Corning Pluronics sacrificial ink	<ul style="list-style-type: none">• Let Corning Pluronics sacrificial ink reach room temperature (RT).
2	Load the syringe	<ul style="list-style-type: none">• Corning Matribot bioprinter• 3 mL syringe of Corning Pluronics sacrificial ink• Corning standard or high precision conical bioprinting nozzles 22-27G	<ul style="list-style-type: none">• Attach the nozzle to the syringe. The reference droplet dispensing protocol uses a 22G nozzle and the reference printing protocol uses a 27G nozzle. Push the plunger to remove air until the Pluronics sacrificial ink reaches approximately halfway through the nozzle.• Place the syringe into the printhead at RT.• Rotate the syringe counterclockwise until the syringe tabs are locked in place.• Adjust the position of the syringe plunger holder arm by navigating on the LCD interface to Prepare Bioprint. Select Raise Plunger to raise the plunger arm to its maximum height, and use Extrude Volume to lower the plunger arm until it aligns with the height of the syringe plunger.• Rotate the syringe plunger holder arm over the syringe plunger.• Extrude 30 μL using the Extrude Volume function on the device's LCD interface in the Prepare Bioprint menu. Hold a lab tissue under the bioprinting nozzle to catch the extruded material.

Step	Title	Material	Description
3	Printing Parameter selection	<ul style="list-style-type: none"> Corning® DNA Studio software 	<ul style="list-style-type: none"> Use Corning DNA Studio software to select parameters based on your application and select Print on the toolbar when complete: <ul style="list-style-type: none"> - See Table 1 for droplet dispensing in a 96-well or 384-well microplate or a multiwell plate using the Droplet function. - See Table 2 for printing three-layered grids into a Petri dish using the Generate function or using the Bioprint function with your own object file. <p>NOTE: The values in Tables 1 and 2 are only a reference point for starting parameters. The actual values needed for your given application will depend on the preparation procedures as well as the print surface.</p> <p>NOTE: Parameter selection can also be performed prior to Pluronic syringe preparation.</p>
4	Machine calibration (manual or automatic)	<ul style="list-style-type: none"> Corning Matribot® bioprinter Petri dish, multiwell plate, or microplate 	<ul style="list-style-type: none"> Place a Petri dish, multiwell plate, or microplate on the printbed. Perform manual or automatic calibration following the software prompts. Perform machine calibration each time a new syringe is placed in the printhead or a new plate type is used. If the printbed is not leveled, perform Automatic bed-leveling (ABL). <p>NOTE: Manual calibration is recommended for 96-well microplates and is necessary for 384-well microplates. Ensure the nozzle tip is placed in the center of the well, since manual calibration results in x, y, and z calibration.</p>
5	Nozzle priming	<ul style="list-style-type: none"> Corning Matribot bioprinter 	<ul style="list-style-type: none"> Immediately before each print, prime the nozzle by extruding 2 to 3 drops. Clean the nozzle before printing using a tissue paper. If any material has gelled at the tip of the nozzle, ensure it is fully extruded prior to starting a print.
6	Printing	<ul style="list-style-type: none"> Corning Matribot bioprinter Petri dish, multiwell plate, or microplate 	<ul style="list-style-type: none"> Press Start to start the printing process. <ul style="list-style-type: none"> - See Figure 1 for reference droplets and Figure 2 for three-layered grid structures. - If the printed structures are not as desired, adjust parameters as needed following the Corning Matribot Bioprinter Parameters (CLS-AN-648). <p>NOTE: The values are only a reference for starting parameters and may need to be adjusted for your specific application.</p>
7	Washing	<ul style="list-style-type: none"> Cold PBS Ice 	<ul style="list-style-type: none"> Corning Pluronic sacrificial ink 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 Corning Pluronic sacrificial ink is removed. If the Corning Pluronic sacrificial ink 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 Corning Pluronic sacrificial ink.
8	Incubation	<ul style="list-style-type: none"> Cell culture medium 	<ul style="list-style-type: none"> After removing the Corning Pluronic sacrificial ink, add the desired medium to the constructs and place in the 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.

Table 1. Recommended settings used for dispensing Corning Pluronic sacrificial ink in a 96-well microplate using the Droplet Print function on the Corning Matribot bioprinter.

Parameters	
Well plate	96-well microplate
Nozzle	0.410 mm (22G)
Temperature printbed	-
Temperature printhead	-
Extrusion rate	30 µL/s
Extrusion volume	35 µL
Retract volume	30 µL
Droplet volume	5 µL
Z-offset	0.5 mm
Extra preflow volume	0 µL
Retract rate	120 µL/s
Postflow stop time	0.3 s
Z-lift	20 mm



Figure 1. Droplets of Corning Pluronic sacrificial ink without cells dispensed in a 96-well microplate, using the parameters in Table 1.

Table 2. Recommended settings used for printing three-layered grids (20 x 20 mm) without cells.

Parameters	
Temperature printbed	-
Nozzle	0.2 mm (27G)
Speed	5 mm/s
Temperature printhead	-
Preflow volume	37.5 μ L
Extrusion rate	2.5 μ L/s
Retract volume	40 μ L
Z-offset	0.1 mm
Extra preflow volume	4.5 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

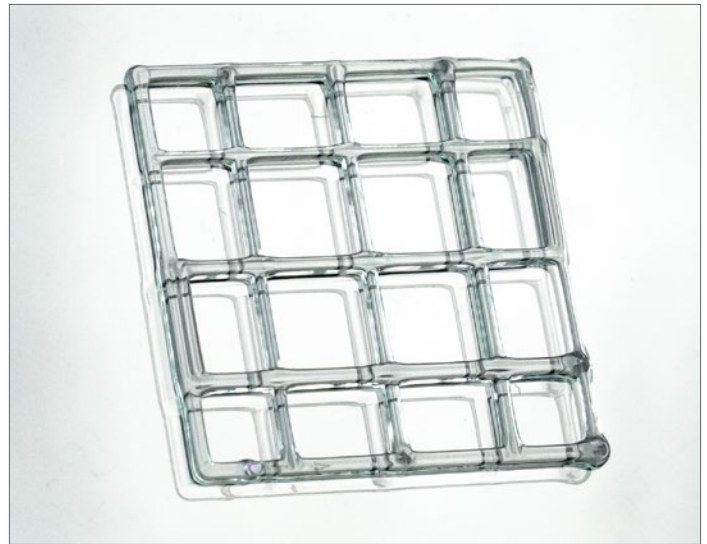


Figure 2. Three-layered grid structures acquired after printing with Corning Plurionics sacrificial ink using the parameters from Table 2.

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Corning Incorporated
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t 800.492.1110
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t 86 21 3338 4338

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t 91 124 4604000
Japan
t 81 3-3586 1996
Korea
t 82 2-796-9500
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t 65 6572-9740
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t 886 2-2716-0338

EUROPE
CEurope@corning.com
France
t 0800 916 882
Germany
t 0800 101 1153
The Netherlands
t 020 655 79 28
United Kingdom
t 0800 376 8660

All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400