

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

CORNING

Protocol

This is a suggested procedure, please 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 Start sacrificial ink using the Corning Matribot bioprinter. Droplet dispensing and grid printing demonstrate the versatility of Corning Start sacrificial ink for simple and complex structures used in many applications for both sacrificial and support 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 Start 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 warmed PBS before starting cell culture.

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 Start sacrificial ink, 2.7 mL/syringe (Corning 6159) or Corning Start sacrificial ink (non-sterile), 2.7 mL/syringe (Corning 6158)
- ▶ Pre-warmed PBS (37°C)
- ▶ Petri dish, multiwell plate, or microplate
- ▶ 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- ▶ Corning standard or high precision conical bioprinting nozzles, 22-27G*
 - Standard conical bioprinting nozzles: 22G-410 μm (Corning 6167), 25G-250 μm (Corning 6166), 27G-200 μm (Corning 6165)
 - High precision conical bioprinting 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

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

Step	Title	Material	Description
1	Load the syringe	<ul style="list-style-type: none">• Corning Matribot bioprinter• 3 mL syringe of Corning Start 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 on the plunger to remove air until the Start ink reaches approximately halfway through the nozzle.• 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.
2	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 multi-well 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 Corning Start sacrificial ink syringe preparation.</p>

Step	Title	Material	Description
3	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). Automatic bed-leveling is disabled as an option until after calibration is performed. <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>
4	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.
5	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. - 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>
6	Corning Start sacrificial ink removal	<ul style="list-style-type: none"> Warm PBS 	<p>Corning Start sacrificial ink cannot be crosslinked. Prior to washing, make sure to crosslink any other bioinks printed to retain their structure.</p> <ul style="list-style-type: none"> For ink removal, wash the bioprinted construct with warmed PBS to liquify and flush away the sacrificial ink from the construct.

Table 1. Recommended settings used for dispensing Corning Start 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	60 $\mu\text{L/s}$
Extrusion volume	20 μL
Retract volume	15 μL
Droplet volume	5 μL
Z-offset	0.3 mm
Extra preflow volume	0 μL
Retract rate	60 $\mu\text{L/s}$
Postflow stop time	0.3 s
Z-lift	20 mm

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	10 mm/s
Temperature printhead	-
Preflow volume	38 μL
Extrusion rate	2.5 $\mu\text{L/s}$
Retract volume	38 μL
Z-offset	0.1 mm
Extra preflow volume	3.5 μL
Infill extrusion multiplier	100%
Retract rate	60 $\mu\text{L/s}$
Extra retract	0 μL
Postflow stop time	0.3 s
Z-lift	3.0 mm

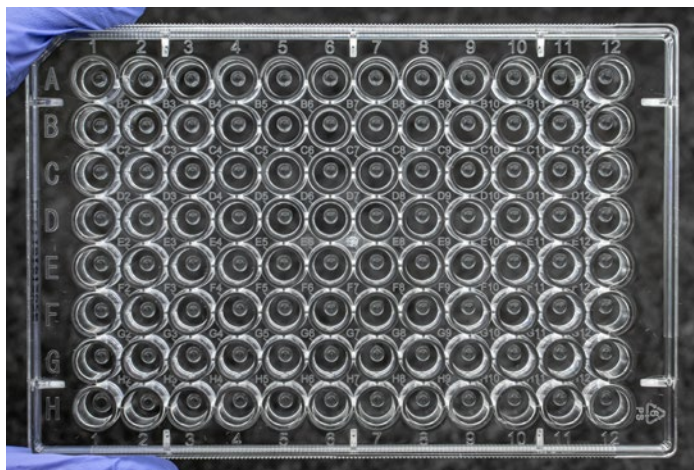


Figure 1. Droplets of Corning Start sacrificial ink without cells dispensed in a 96-well microplate, using the parameters in Table 1.



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

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