

# Corning® Matribot® Bioprinter

CORNING

## Demo Protocol Experiment 2:

Mimicking Corning Matrigel® matrix dispensing for droplet arrays in 24-well plates using Corning Pluronics sacrificial ink

### Introduction

The aim of this protocol is to provide instructions for dispensing arrays of droplets of Corning Pluronics sacrificial ink using the Corning Matribot bioprinter. Droplets of Corning Pluronics sacrificial ink can mimic the handling and dispensing process of 9 mg/mL Corning Matrigel matrix solution. This document covers dispensing parameters and procedures for dispensing pairs of droplets into 24-well plates. This protocol was optimized for Pluronics sacrificial ink diluted to 25% with water.

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

### Materials

- ▶ Corning Pluronics sacrificial ink, 2.7 mL/syringe (Corning Cat. No. 6157)
- ▶ Corning Matribot bioprinter (Corning Cat. No. 6150)
- ▶ Corning standard conical bioprinting nozzles, 22G-410 µm (blue; Corning Cat. No. 6167)
- ▶ Costar® TC-treated 24-well plate (Corning Cat. No. 3524)
- ▶ Purified water, 5 mL pre-chilled at 4°C
- ▶ 3 mL syringe with BD Luer-Lok™ tip (BD Cat. No. 309657)
- ▶ Syringe Luer-Lok cap (BD Cat. No. 408531)
- ▶ 15 mL conical tube
- ▶ Serological pipet or pipet tips pre-chilled at 4°C

### Protocol

This protocol has been optimized for use with the Corning Matribot bioprinter, which has a cooled printhead. However, clogging of Pluronics sacrificial ink at the nozzle tip may still occur. Set the printhead temperature prior to loading the Pluronics sacrificial ink syringe as recommended in Step 1. Keep the Pluronics sacrificial ink refrigerated or on ice until loaded into the pre-cooled Corning Matribot bioprinter printhead. When preparing 25% Pluronics sacrificial ink solution, pre-chill all plastics coming into contact with the Pluronics sacrificial ink such as pipet tips.

Step	Title	Material	Description
1	Set Printhead and Printbed temperatures	<ul style="list-style-type: none"><li>• Corning Matribot bioprinter</li><li>• Computer with Corning DNA Studio software</li><li>• 24-well plate</li></ul>	<ul style="list-style-type: none"><li>• Connect the printer to the computer using the provided USB cable.</li><li>• Turn on the Matribot bioprinter using the power switch.</li><li>• Open the latest version of Corning DNA Studio.</li><li>• Connect to the printer.</li><li>• In the Corning DNA Studio software, select Utilities from the Tools drop down menu. In the Utilities window, select the Temperature tab. Set the Printhead temperature to 4°C and Printbed temperature to 37°C by entering the desired temperature values and selecting the slidebars to activate temperature control.</li><li>• Attach the standard thermal insulator.</li><li>• Place a 24-well plate on the printbed.</li></ul>
2	Set dispensing parameters	Corning DNA Studio software	<ul style="list-style-type: none"><li>• Use Corning DNA Studio software to create a Droplet project.<ul style="list-style-type: none"><li>- Open a Droplet project.</li><li>- In the Surface tab, select a Corning COSTAR 24-well plate. Select the two bottom rows of wells.</li><li>- Enable Droplet Array feature and select the pattern of 2 droplets.</li><li>- In the Printer tab, set Printbed temperature to 37°C.</li><li>- In the Printhead tab, set Bioink to Corning Pluronics sacrificial ink 25% and confirm that the Printhead parameters match the parameters in Table 1.</li></ul></li><li>• Select Print on the software toolbar when complete. Click OK on the pop-up window to proceed to the printing page.</li></ul>

Step	Title	Material	Description
3	Prepare 25% Pluronics solution	<ul style="list-style-type: none"> <li>• Corning® Pluronics sacrificial ink</li> <li>• Conical tube</li> <li>• Purified water</li> <li>• 3 mL syringe</li> <li>• Syringe Luer-Lok cap</li> <li>• Ice bath</li> </ul>	<ul style="list-style-type: none"> <li>• Prepare 25% Pluronics solution by diluting Corning® Pluronics sacrificial ink, which contains 40% Pluronics, in purified water.</li> <li>- Prior to starting, place on ice a Corning Pluronics sacrificial ink syringe, a conical tube, a tube containing purified water, and a capped 3 mL syringe with the plunger removed.</li> <li>- Use the plunger on the Corning Pluronics sacrificial ink syringe to push the ink into the empty 15 mL conical tube until the Pluronics sacrificial ink reaches the desired fill line (e.g., 2 mL).</li> <li>- Using a pre-chilled serological pipet or pipet tip, transfer the appropriate amount of cold purified water (e.g., 1.2 mL) into the same tube to dilute the Pluronics sacrificial ink to a 25% solution. Slowly mix to form a homogenous solution taking care not to let the solution warm. As the solution warms, the viscosity increases, complicating mixing.</li> <li>- Transfer 2.7 mL of 25% Pluronics solution to the prepared 3 mL syringe and place the plunger into the syringe. While holding the plunger in place, flip the syringe with the cap end up and replace the syringe cap with a 22G nozzle. Push on the plunger to remove air until the 25% Pluronics solution reaches approximately halfway through the nozzle.</li> </ul>
4	Load the syringe	<ul style="list-style-type: none"> <li>• 3 mL syringe of 25% Corning Pluronics solution</li> <li>• Standard conical bioprinting nozzle, 22G-410 µm</li> </ul>	<ul style="list-style-type: none"> <li>• Remove air bubbles from the syringe by tapping the syringe.</li> <li>• After the printhead has reached 4°C, place the loaded syringe into the pre-cooled printhead.</li> <li>• Rotate the syringe counterclockwise until the syringe tabs are locked in place.</li> <li>• 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.</li> <li>• Rotate the syringe plunger holder arm over the syringe plunger.</li> </ul>
5	Machine Calibration (automatic)	<ul style="list-style-type: none"> <li>• Corning Matribot bioprinter</li> <li>• Corning DNA Studio software</li> </ul>	<ul style="list-style-type: none"> <li>• 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 printing nozzle to catch the extruded material.</li> <li>• In Corning DNA Studio, click Calibrate and select Automatic Calibration.</li> <li>• Perform automatic calibration by following the steps displayed in Corning DNA Studio.</li> <li>• Perform machine calibration each time a new syringe is placed in the printhead or a new plate type is used.</li> </ul>
6	Nozzle priming	<ul style="list-style-type: none"> <li>• Corning Matribot bioprinter</li> <li>• Corning DNA Studio software</li> </ul>	<ul style="list-style-type: none"> <li>• Immediately before each print, prime the nozzle by extruding 2 to 3 drops. Priming can be performed either by selecting the droplet icon in the software printing window or by extruding 1 µL increments by using the Extrude Volume function on the LCD interface. If any material has gelled at the tip of the nozzle, ensure it is fully extruded prior starting a print.</li> </ul> <p><b>NOTE:</b> If the system has been idle for an extended period, the Pluronics sacrificial ink in the nozzle can dry or gel causing it to clog. If this occurs purge the nozzle by extruding 30 to 60 µL of the Pluronics sacrificial ink, or until the gelled part is extruded. If it is not possible to extrude the gelled Pluronics sacrificial ink, replace the clogged nozzle with a new nozzle. Always ensure the nozzle is fully primed prior to printing.</p>
7	Printing	<ul style="list-style-type: none"> <li>• Corning Matribot bioprinter</li> <li>• Corning DNA Studio software</li> </ul>	<ul style="list-style-type: none"> <li>• In Corning DNA Studio, press Start to begin dispensing droplets.</li> <li>• After the dispensing process is finished, close the Printing window by clicking the (x) in the corner of the bottom window. In the Surface tab, select the top two rows of wells.</li> <li>• Select Print on the software toolbar when complete. Click OK on the pop-up window to proceed to the printing page. and repeat Step 6 to prime the nozzle.</li> <li>• Start the second dispensing process.</li> </ul>

**Table 1.** Recommended printhead settings used for dispensing 25% Corning® Pluronic solution using the Droplet Print feature on the Corning Matribot® bioprinter.

Printhead Settings	25% Solution
Temperature printbed	37°C
Temperature printhead	4°C
Extrusion rate	20 µL/s
Extrusion volume	6 µL
Retract volume	3 µL
Droplet volume	3 µL
Z-offset	0 mm
<b>Advanced</b>	
Extra preflow volume	0 µL
Retract rate	20 µL/s
Postflow stop time	0 s
Z-lift between wells	30 mm

**NOTE:** This is only a recommended reference of starting parameters. The actual values needed to print will vary depending on the preparation procedures (amount of Pluronic sacrificial ink and actual temperature of the Pluronic sacrificial ink) as well as the print surface. If printing does not begin right away, it is most likely because the printhead or printbed has not yet reached the temperature set-point.

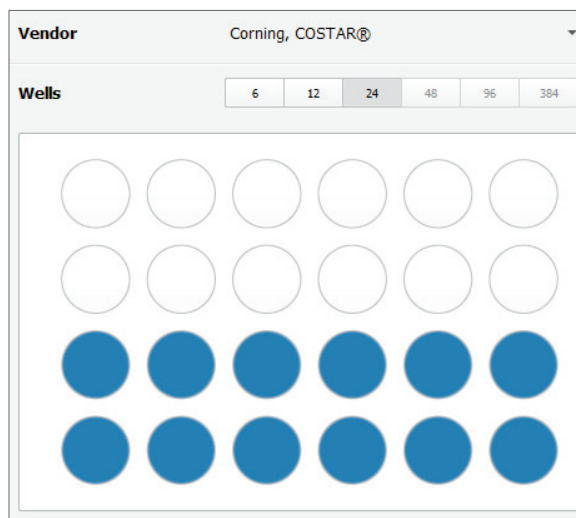


Figure 1. Well plate selection for the first part of the experiment.

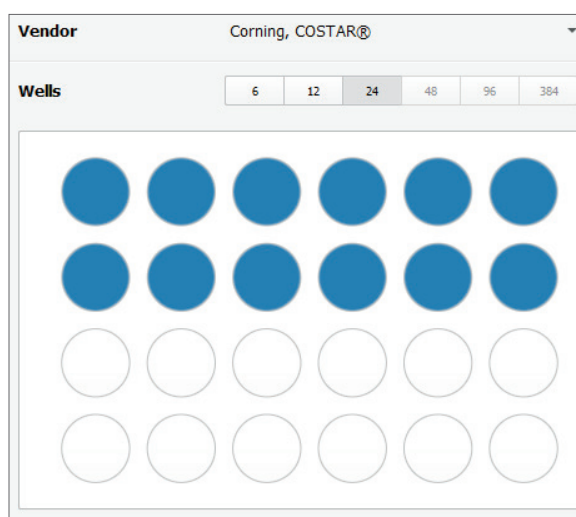


Figure 2. Well plate selection for the second part of the experiment.

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