

Corning® Matribot® Bioprinter

CORNING

Demo Protocol Experiment 1:

Mimicking Corning Matrigel® matrix dispensing into 96-well microplates using Corning Pluronics sacrificial ink

Introduction

The aim of this protocol is to provide instructions for dispensing droplets of 25% Corning Pluronics solution using the Corning Matribot bioprinter. Droplets of Corning Pluronics sacrificial ink can mimic the dispensing process of 9 mg/mL Corning Matrigel matrix. This document covers dispensing parameters and procedures for dispensing into a 96-well microplate. This protocol was optimized for Corning Pluronics sacrificial ink diluted to 25% with water.

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

Materials

- Corning Pluronics sacrificial ink, 2.7 mL/syringe (Corning 6157)
- Corning Matribot bioprinter (Corning 6150)
- Corning standard conical bioprinting nozzles, 22G-410 µm (blue; Corning 6167)
- Corning TC-treated 96-well microplate (Corning 3596)
- Purified water, 5 mL pre-chilled at 4°C
- 3 mL syringe with BD Luer-Lok™ tip (BD 309657)
- Syringe Luer-Lok cap (BD 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 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">• Corning Matribot bioprinter• Computer with Corning DNA Studio software• 96-well microplate | <ul style="list-style-type: none">• Connect the printer to the computer using the provided USB cable.• Turn on the Matribot bioprinter using the power switch.• Open the latest version of Corning DNA Studio.• Connect to the printer.• In 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.• Attach the standard thermal insulator.• Place a 96-well microplate on the Printbed, and press it into place so that it is flush with the Printbed. |
| 2 | Set dispensing parameters | <ul style="list-style-type: none">• Corning DNA Studio software | <ul style="list-style-type: none">• Use Corning DNA Studio software to create a Droplet project.<ul style="list-style-type: none">- Open a Droplet project.- In the Surface tab, select a Corning COSTAR 96-well microplate.- In the Printer tab, set Printbed temperature to 37°C.- In the Printhead tab, set Bioink to Corning Pluronics Sacrificial Ink 25%, and confirm that the Printhead parameters match the parameters in Table 1.• Select Print on the software toolbar when complete. Click OK on the pop-up window to proceed to the printing page. |

| Step | Title | Material | Description |
|------|--------------------------------|--|---|
| 3 | Prepare 25% Pluronics solution | <ul style="list-style-type: none"> Corning® Pluronics sacrificial ink Conical tube Purified water 3 mL syringe with BD Luer-Lok tip BD Luer-Lok cap Ice bath | <ul style="list-style-type: none"> Prepare 25% Pluronics solution by diluting Corning Pluronics sacrificial ink, which contains 40% Pluronics, in purified water. 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. Take note of the ink volume in the syringe based on the syringe graduation lines (e.g., 2.7 mL). Use the plunger on the Corning Pluronics sacrificial ink syringe to push the ink into the empty 15 mL conical tube until the Pluronics ink remaining in the syringe reaches the desired graduation line on the syringe (e.g., 0.7 mL if dispensing 2 mL from 2.7 mL initial volume). 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 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. 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. |
| 4 | Load the syringe | <ul style="list-style-type: none"> 3 mL syringe of 25% Corning Pluronics solution Standard conical bioprinting nozzle, 22G-410 µm | <ul style="list-style-type: none"> Remove air bubbles from the syringe by tapping the syringe. After the printhead has reached 4°C, place the loaded syringe into the pre-cooled printhead. 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 printing nozzle to catch the extruded material. |
| 5 | Machine calibration (manual) | <ul style="list-style-type: none"> Corning Matribot bioprinter Corning DNA Studio software | <ul style="list-style-type: none"> If there is a lid on the plate, remove it before starting calibration. In Corning DNA Studio, click Calibrate, and select Manual Calibration. Perform manual calibration by aligning the nozzle tip in the center of well H1 using the arrows and up/down buttons on the software window. Place the nozzle as close to the printing surface as possible without touching it. It is recommended to start with larger step sizes (5 to 10 mm) and to decrease step size as the alignment gets closer, finishing with the 0.1 mm step size. When the desired alignment has been reached, select Calibrate in the software window. Perform machine calibration each time a new syringe is placed in the printhead or a new plate type is used. |
| 6 | Nozzle priming | <ul style="list-style-type: none"> Corning Matribot bioprinter Corning DNA Studio software | <ul style="list-style-type: none"> 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. <p>NOTE: 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 the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with ink prior to printing.</p> |
| 7 | Printing | <ul style="list-style-type: none"> Corning Matribot bioprinter Corning DNA Studio software | <ul style="list-style-type: none"> In Corning DNA Studio, press Start to begin dispensing droplets. If the dispensed droplets are not placed in the center of wells, repeat the manual calibration procedure. If the printed droplets are not as desired, adjust the extrusion volume up or down by 1 µL to extrude more or less material. See Figure 1 for reference droplets. |

Table 1. Recommended printhead settings used for dispensing 25% Corning Pluronics solution using the Droplet Print function 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 |
| Advanced | |
| Extra preflow volume | 0 µL |
| Retract rate | 20 µL/s |
| Postflow stop time | 0 s |
| Z-lift between wells | 30 mm |

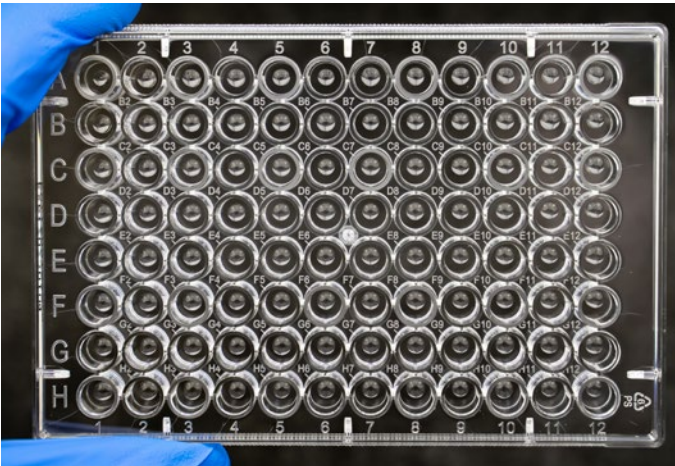


Figure 1. Droplets of 25% Corning Pluronics solution acquired after dispensing with the parameters from Table 1.

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 Pluronics sacrificial ink and actual temperature of the Pluronics 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.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use or general laboratory use only.* Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. These products are not intended to mitigate the presence of microorganisms on surfaces or in the environment, where such organisms can be deleterious to humans or the environment. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications. *For a listing of US medical devices, regulatory classifications or specific information on claims, visit www.corning.com/resources.

CORNING

Corning Incorporated
Life Sciences
www.corning.com/lifesciences

NORTH AMERICA
t 800.492.1110
t 978.442.2200

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
Chinese Mainland
t 86 21 3338 4338

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

EUROPE
CSEurope@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