NanoSurface **Plates**

Nanoscale Topography Promotes Physiological Structure and Function

User Manual

Last revised:
October 1, 2024

curi bio

Terms and Conditions

Release of Liability

This document is provided to you “as is” for your information and is to be used at your own risk. Curi Bio, Inc. assumes no responsibility for any inaccuracies (technical, typographical, or otherwise). Curi Bio, Inc. reserves the right to change information that is contained in this document at any time.

Curi Bio, Inc., without limitation makes no representations, warranties, or agreements (either express or implied). Nor does Curi Bio make warranties or conditions of fitness for a particular reason or for the performance of any product referenced in this guide (either express or implied). Curi Bio, Inc. does not in any way guarantee or represent that you will obtain satisfactory results from using our products as described herein. You assume all risk in connection with your use of our products.

Conditions of Use

Curi Plates are licensed for life science research only and may not be used for any other purpose. No other right, express or implied, is conveyed by the sale of our products. You are not permitted to use our products in humans, in clinical trials or for diagnostic purposes involving human subjects, for any medical or therapeutic use, nor for any purpose in contravention of any applicable law or regulation. You are responsible for understanding and safely performing the protocols described within this guide. Curi Bio, Inc. does not guarantee any results you may achieve. These protocols are provided as Curi Bio, Inc. recommendations to you and does not constitute a guarantee of success.

Technical Support

Contact support@curibio.com for any assistance.

CONTENTS

Section 1: General Information

Thank You

Thank you for purchasing Curi Plates. Our unique line of biomimetic culture dishes enhance your experiments by replicating the *in vivo* extracellular environment. Curi Plates are manufactured with a high-precision, high-consistency nanopatterned surface and will provide a reliable and scalable platform for your cell culture needs.

Handling

Curi Plates have been tissue-culture treated except for coverglasses (see *note below*).

⚠ **Note**: Before using the Curi coverglasses, it is recommended that you sterilize them. This can be accomplished by autoclaving the coverglasses or by briefly submerging them in 70% ethanol:water (sonication recommended), followed by rinsing with ultrapure and sterile water, then drying in a sterile cell culture hood or under vacuum.

While the nanopatterned polymer is durable, Curi advises against making direct contact with the surface. For best results, avoid touching the surface with items such as pipette tips, tweezers, etc. Similarly, take extra care in handling multi-well plates to avoid undue damage to the thin glass bottom.

Nanopattern Composition and Structure

The nanopatterns are formed using a very thin layer of polymer spread uniformly across a coverslip-quality glass to form the cell culture substrate. The total thickness of this substrate is No. 1.5 (0.175 $\pm$ 0.015 mm). A single large sheet of the substrate is attached to the bottom of the plate, with each well having a total thickness equivalent to No. 1.5 (0.175 $\pm$ 0.015 mm).

For coverslips, you can identify the patterned side by reflecting light off the surface. Alternatively, you can place a droplet of sterile diH_2O and tilt the surface. The droplet will only run in one direction if it is on the nanopatterned side.

The nanopattern is made using an optically transparent proprietary polymer. The modulus of the polymer is approximately 7 MPa and has a refractive index of 1.51. It has excellent transmissivity above 350 nm to IR (>1.2 µm).

The nanopattern structure consists of submicron grooves and ridges, shown in **Fig. 1**.

Nanopattern Composition and Structure (Continued)

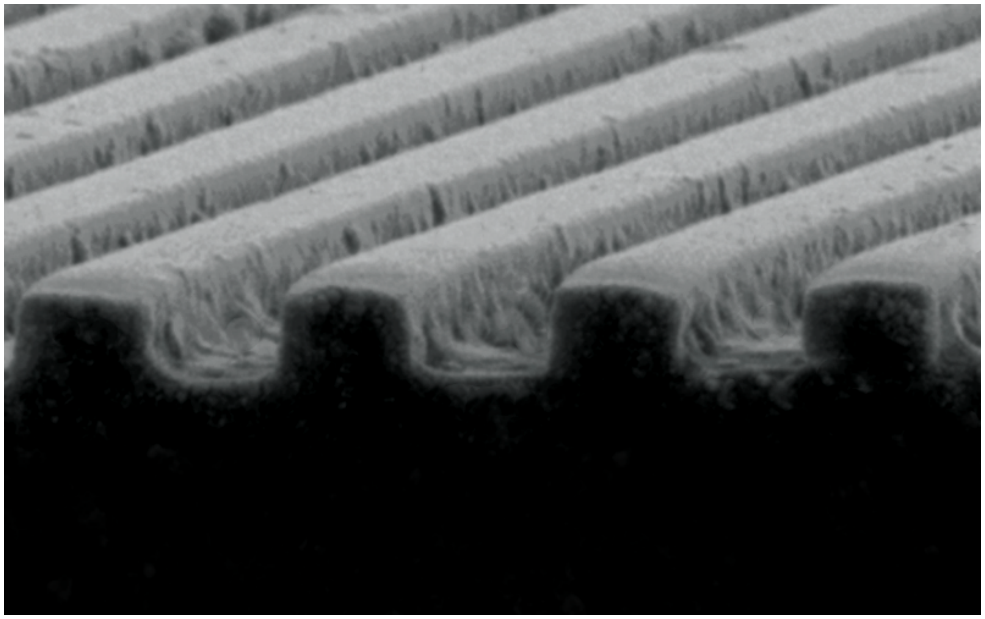

Figure 1: *A scanning electron micrograph of the nanopattern showing the surface and crosssection with the approximate dimensions of the nanopattern structure.*

Section 2: Coating with Different Extracellular Matrix Proteins

Important Note

For best results, Curi recommends coating the surface with extracellular matrix (ECM) proteins. Much like standard cell cultureware, all NanoSurface Plate formats **except for the coverglass products** are pre-treated with oxygen plasma to facilitate cell adhesion and functionalization. However, Curi Bio recommends that you use a much lower concentration of ECM proteins. The high concentrations that are commonly used may obscure the nanopattern and negate its function. Curi also recommends that you allow for longer adsorption times when incubating proteins on the culture surface.

Suggested Protocols

In this section, you can find our suggested protocols and specific workflow adjustments for some common ECM proteins. If you have any specific protocol related question, please don't hesitate to reach out to support@curibio.com.

1. Prepare your ECM protein.
    a. ***Fibronectin:*** Dilute your fibronectin stock to a working concentration. Curi recommends using a concentration of 5 µg/mL, though concentrations up to 50 µg/mL have been used successfully. We highly recommend diluting your fibronectin in PBS that contains Mg^{2+} and Ca^{2+}.
    b. ***Fetal Bovine Serum (FBS):*** You can add FBS directly to Plates.
    c. ***Matrigel:*** Prepare 1:60 Matrigel™ dilution in culture medium or PBS.
    d. ***Collagen:*** Prepare a sterile solution of 5 µg/mL collagen in 0.02 M acetic acid. An HCL solution of 0.01 M can be used or as specified by the manufacturer.
    e. ***Gelatin:*** Prepare a sterile 0.2% gelatin solution in PBS.
    f. ***Laminin:*** Dilute stock to a working concentration. Curi recommends a concentration of 5 µg/mL, and highly recommends diluting your laminin in PBS that contains Mg^{2+} and Ca^{2+}.
2. Cover the bottom of the wells with the ECM protein solution. If the drop is difficult to spread, you may have to use a higher volume of solution to cover the entire surface. Please note that:
    a. For single well dishes, there is no need to treat the flat plastic edges of the dish.
    b. For 384-well plates, sometime air bubbles may prevent your protein coating from fully covering the surface of the wells. To

resolve that, you will need to centrifuge the plates after protein coating to ensure that your protein solution is fully covering the surface of the wells. You can simply use your microplate swinging bucket rotor attachment on your centrifuge to ensure proper ECM protein coating across the entire surface. After correctly weighing and placing your plates in the centrifuge, exposing the plates to a short pulse of 15 seconds at maximum speed in the centrifuge should allow your ECM protein to fully coat the surface of the well. Inspect your plate when removing from the centrifuge to ensure the protein is adequately distributed.

c. **Incubate the solution overnight** at 37°C/5% CO_2 in sterile conditions. A cell culture incubator works well.

3. After incubation, aspirate the solution and immediately seed your cells at your desired concentration.

Section 3: **Cell Seeding**

Important Note

Due to the highly variable nature of cell attachment protocols, it is difficult to provide a one-size-fits-all protocol for cell seeding. In general, Curi recommends starting with protocols verified for a given cell type on conventional surfaces and modifying as needed. In some cases, increasing the time of initial seeding or adding 5% FBS to your medium can facilitate better attachment to the nanopatterned surfaces.

Cell Seeding Example

IPSC-DERIVED CARDIOMYOCYTES

⚠ ***Note:*** this protocol assumes you have already coated the dish with your protein of choice, as outlined in Section 2.

1. Seeding will be based on the specific plate product you are using:
    a. For 35 mm dishes, use a two-step procedure (as shown in **Fig. 2**):
        1) Pipette 0.6 mL of cell solution onto the nanopatterned area of the plate; take care not to spread the drop onto the polystyrene dish (see figure below).
        2) Allow the cells to attach for 6-8 hours (overnight if possible) in the cell culture incubator (37°C, 5% CO_2).
    b. For 384-well plates, you will need to follow the same centrifuging protocol explained in ”suggested protocols” section of this manual. This is to ensure that your cells are fully covering the surface of the wells.
    c. For every other NanoSurface Plate formats, use the same procedure that you would on conventional cultureware.
2. Maintenance: Curi recommends half-medium changes. This will allow soluble growth factors to remain and will also lower the chance of pressing the pipette tip into the sample/cell surface, which can damage the cells.

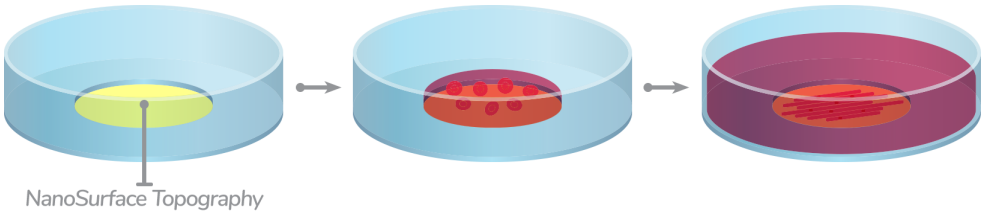

Figure 2: Two-step procedure for coating 35 mm dishes (used in this example image). Start with a small droplet (middle image) and allow the cells to attach. After the attachment period, fill the dish or well with a working volume of culture medium (right image).

Representative images of iCell® Cardiomyocytes plated on Curi 96-well plates at various stages of development are included in **Fig. 3**, **Fig. 4**, and **Fig. 5**.

Figure 3: *Day 4 bright field images at 10X (left) and 20X (right), where the nanopattern is visible.*

Figure 4: *Day 14 bright field images at 10X (left) and 20X (right).*

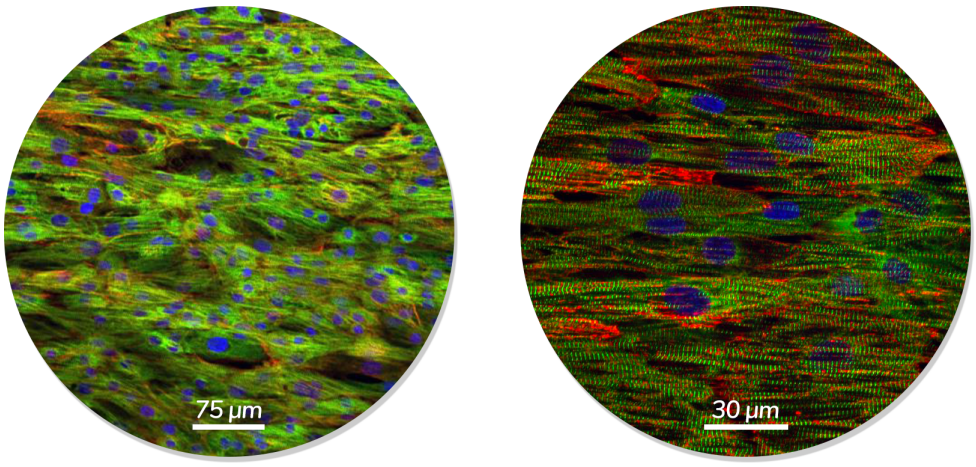

Figure 5: *Day 18 confocal images showing expression of titin (green), actin (red), nuclei (blue).*

Section 4: **Technical Specifications**

Product Specifications

Product Type	Product Code	Pattern Growth Area (cm^2)	Total Well Volume (µL)	Working Volume (µL)
96-Well Plate*	ANFS-0096	0.33	360	200
384-Well Plate*	ANFS-0384	0.081	133	15–110

**ANSI/SLAS compliant.*
All numbers approximate and subject to revision.

More Resources

For more examples of product use, visit www.curibio.com/publications to explore relevant publications.

If you have questions or concerns, please contact support@curibio.com for assistance.

FAQs

What is the substrate thickness on Curi Plates and is the substrate transparent?	The substrate thickness (glass plus polymer coating) is specified at 160–190 microns (0.16–0.19 mm). This is the same thickness specification as a No. 1.5 coverglass. The polymer has an index of refraction comparable to that of glass.
Some Curi Plates come in two different topographies. Which one should I use for my application?	Our coverglasses and 35 mm dishes come in two different topographies: (1) unpatterned ”flat” and (2) Curi topography. Both formats have a polymer substrate that is softer than conventional glass or plastic cultureware. As a result, the softer substrates are more representative of the mechanical properties of the tissue microenvironment. The Curi substrate possesses submicron grooves with a feature size that is comparable to the collagen microenvironment in the body. As a result, the pattern is also optimized to be in the size of the focal adhesion of cells and facilitate the alignment of adherent cells. Our customers usually use flat substrates either for their improved mechanical properties (softer substrate) or as a control sample for NanoSurface topography to show the differences in the functionality of aligned and non-aligned tissue.
Are all Curi Plate formats tissue culture treated?	All of our plates are TC-treated and ready for use.
Can I grow cells directly on Curi Plates or do I need to do ECM protein coating?	We recommend coating the surface with the extracellular matrix protein you typically use in your cell culture workflow (collagen, fibronectin, gelatin, Matrigel, etc.). We also have a suggested concentration in our manual for protein coating of our substrates using some of these proteins. Before starting your experiments, make sure to check our manuals where you can find the details of the protocols for each protein coating.
Is there a suggested cell seeding density for iPSCs?	For most commercially available cell lines, the cell seeding density used on conventional cultureware works with NanoSurface Plates as well. If you are using your own cell line, our support team can provide you some suggestions on how to design seeding density optimization experiments. If you require additional support, please contact support@curibio.com so that our applications team can provide you with the relevant suggested protocol.

Can I use the same cell seeding densities that I use for conventional cultureware?	Yes, we recommend that you seed cells using the same density that you use with conventional plates.
What are the plate dimensions? Are NanoSurface Plates compliant with standard workflows?	NanoSurface Plates are ANSI/SLAS/SBS compliant and can be used in most standard assay workflows. For dimension details of our plate formats, please contact us at support@curibio.com.
How do you determine the direction of the nanopattern without looking under microscope?	The pattern runs parallel with the longitudinal direction of the plate.
Can I use the same volume (of medium) as usual?	Yes, please refer back to Table 1 for more detailed suggestions. Also, the volume and frequency of cell medium changes is the same as what is recommended for conventional cultures of your cells of choice.
Do you have suggested cell seeding protocols for different cell types?	As with any cultureware, optimal seeding density on NanoSurface Plates can vary according to cell type and the purpose of the experiment. However, we recommend researchers use the same cell density that they would use for conventional cultureware. For specific applications, please contact us at support@curibio.com.
What is the shelf life of NanoSurface Plates?	We recommend using your NanoSurface Plates within 2 years of purchase.
What is the storage condition for NanoSurface Plates?	We recommend storing NanoSurface Plates where it is protected from light and under climate controlled conditions (Ex. 20-25 degrees Celsius, 30-50% humidity).
More Questions?	If you have questions or concerns, contact support@curibio.com for assistance.