THE HANDBOOK FOR

NANOCULTURE PLATE (NCP) Ver.2

Product type		Name	Cat. #	Size
96-well	Low-Binding	NanoCulture Plate MH Pattern Low-Binding 96-well Ver.2	NG-PLH9002	2 plates
			NG-PLH9010	10 plates
	High-Binding	NanoCulture Plate MH Pattern High-Binding 96-well Ver.2	NG-PHH9002	2 plates
24-well	Low-Binding	NanoCulture Plate MH Pattern Low-Binding 24-well Ver.2	NG-PLH2002	2 plates
			NG-PLH2010	10 plates
	High-Binding	NanoCulture Plate MH Pattern High-Binding 24-well Ver.2	NG-PHH2002	2 plates

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INTRODUCTION

Description

NanoCulture system uses a special material and pattern for three-dimensional (3D) cell culture without any gel matrix or matrix-coating on the cultureware. This system is simple and easy to work with like conventional cell culture technique. Topography of nanoscale pattern on the culture surface helps cells to form spheroids. [Patent filed]

PRODUCTS AND STORAGE

Table 1: Area, Medium Volume & Cell Seeding No:	**NanoCulture Plate Ver.2**	
	24-well	96-well
Area (mm^2)	193	34
*Medium volume	1.5 mL	100 µL
*Cell seeding No. (cells per dish/well)	60,000	10,000

*__Note:__ *Medium volume and cell seeding number can be modified/optimized depending on your experiment.*

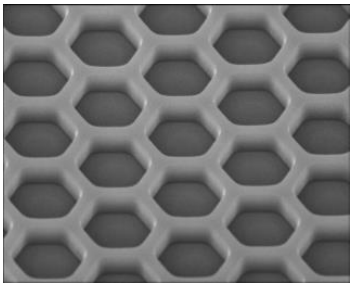
Cell adhesion type

Low-binding (L) and High-binding (H)

Most of the cases, L type plate is suitable for cancer cells. H type plate is recommended for primary adipocyte or hepatocyte and stem cells which are easily detach from culture surface and condense in the well center.

Micro-Pattern type

Micro Honeycomb (MH)



Micro Honeycomb (MH)

Unpacking instruction

Please pay special attention when opening the plastic packaging of products, and avoid putting any pressure or jabbing the bottom film of the cultureware at any occasion. The film is very thin, and nanostructure of the film is very delicate.

Storage and expiration date

The cultureware should be stored at the dark place to avoid a direct light exposure, especially UV light. For the best results, please use the cultureware before the expiration date.

Limitations and precautions

1. The cultureware is for research use only.

2. Please note that the bottom film microstructure might be damaged with pipette tips or any sharp object. To avoid touching the bottom film of the well, please pipette liquid in the well by touching the sidewall of the well and keeping the pipette tip 3-5 mm above the well bottom.

3. The capacity of the cell adhesion and spheroid formation might decrease when confluent cells are used.

PROCEDURE AND TIPS FOR 3D CELL CULTURE

Material

- Sterile PBS (-)
- Cell dissociation reagent i.e. Trypsin/EDTA
- Growth medium: Researchers may work with their own medium but please follow the following Note carefully. ***NOTE:*** *10% serum is recommended for culture media.* ***Serum has to be heat-inactivated.*** *Researchers may optimize the serum percentage depending on their experiments. However, cells may not form spheroids in too less or excess serum containing medium. A Chemically defined media may be allowed. If serum-free medium is used, the cell adhesion might decrease. If you can't get spheroids, please try to use our special media or medium supplement (see related product list on last page of the handbook), or add 0.5 to 1% (final concentration) of Corning Matrigel™ into the media. It will help the cells to form spheroids.*
- Others: Equipment & reagents for conventional 2D cell culture.

Experimental outline

Experimental outline provides an overview of the major steps in the protocol for 3D cell culture. Please follow the following steps:

- Pre-culture of cell that you want to evaluate
- Pre-wetting of the NCP bottom surface
- Preparation of cell suspension
- 3D Cell culture

Detailed Protocol for 3D cell culture

All steps should be performed in the tissue culture hood as for conventional 2D cell culture experiments.

Pre-culture of cell you want to evaluate

1. Grow cells you want to evaluate in advance using conventional 2D cell culture technique.

2. Avoid using confluent cells. Cell attachment on the NCP may take longer time if confluent cells are used.

Pre-wetting of the bottom surface

Air bubbles can form on the well bottom because NCP is made of a plastic with low wettability in combination with the nano-imprinted pattern. To remove any air bubbles it is highly recommended to follow the ***pre-wetting*** *step before seeding the cells.*

3. Add 50% of the culture medium (only) into each well. Then centrifuge the NCP at 1000 x g for 3-5 min to remove the bubble from the well for 96-well plate. For 24-well plate, pipetting is enough to remove the bubbles.

4. Incubate plate for 15-30 min at 37◦C in tissue culture incubator or room temperature in the tissue culture hood for removing micro-bubbles from the microstructure while preparing the cell suspension for seeding.

Preparation of cell suspension for seeding on the NCP

5. Trypsinize the maintained cells and then re-suspend the cells until you reach single cell dispersion. ***NOTE:*** *If cells are dispersed well, uniform spheroid will be formed.*

6. Count the number of cells in the suspension.

7. Adjust to the adequate cell density with culture medium for your experiment. ***NOTE:*** *The density of the cell suspension should be adjusted to 2 times of final cell seeding density.*

Cell culture on the NCP

8. Seed the cells with 50% of final media volume of the cell suspension, which has been prepared in step 7, into each well. Then keep the plate on the bench at room temperature for 10-15 minutes until cells adhere to the bottom film. ***NOTE:*** *If you immediately place the plate in incubator after cell seeding, medium convection current will affect to cells/spheroids distribution that may lead to irreproducibility of experiment. And after cell seeding, please avoid shaking the plate.*

9. Optional: For 96-well and 24-well plate, it is highly recommended adding sterilized water or PBS to the gutter surrounding the plate to prevent medium evaporation. Or covering the top of the plate with a plate sealing tape **(i.e. NUNC cat. #241205)** is effective.

10. Place the NCP plate in the CO2 incubator at 37◦C. Take care not to shake the plate for uniform spheroid formation. ***NOTE:*** *Cells start to form spheroid from day 1 to day 3. If anyone needs to change the medium please change the half medium carefully.*

RELATED PRODUCT INFORMATION

Hypoxia Probe: NC-LOX-1s

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