THE HANDBOOK FOR NANOCULTURE® PLATE

(96well)

Cat. #

NCP-LS96-2, NCP-LS96-10
NCP-HS96-2, NCP-HS96-10
NCP-LH96-2, NCP-LH96-10
NCP-HH96-2, NCP-HH96-10
NCP-LSH96-2

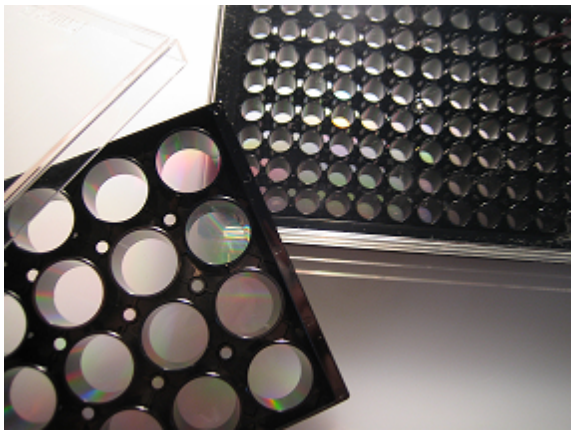


TABLE OF CONTENTS

INTRODUCTION

Precautions for use

Thank you for purchasing a NanoCulture® Plate (NCP). It is recommended that users read all instructions before use of NCP.

Description

NanoCulture® Plate system uses a novel material developed for the three-dimensional cell culture without use of either gel matrix or matrix-coating on the culture plates. This system simply enables the three-dimensional cell culture using conventional cell culture techniques. An uneven surface at nanoscale pattern on the culture plate makes the spheroid formation possible. [Patent filed]

Application

NanoCulture® trial plate can be used for the three-dimensional culture of a variety of cell types including tumor cells. You can see the achievements of our 3D culture system at our website (www.scivax.com/cell) and/or contact us. Please refer to the page 7 for our contact information.

PRODUCTS AND STORAGE

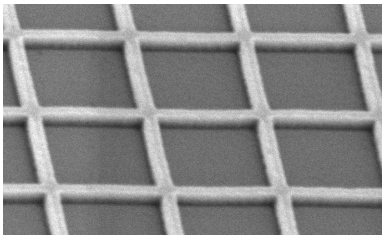
NCP has a particular pattern on the film that composes a bottom of the plate. Culture condition of your own cells on the NCP should be optimized using both MS and MH pattern.
NCP-H is more adhesive type plate for the cell forms spheroid that is easy to detach from NCP-L. Since some cells cannot form spheroid in NCP-H, please refer to our website (www.scivax.com/cell) or contact us for the details.

Cat. #	Pattern type	Attachment type	well	Quantity
NCP-LS96-2	MS	L	96	2
NCP-LS96-10	MS	L	96	10
NCP-HS96-2	MS	H	96	2
NCP-HS96-10	MS	H	96	10
NCP-LH96-2	MH	L	96	2
NCP-LH96-10	MH	L	96	10
NCP-HH96-2	MH	H	96	2
NCP-HH96-10	MH	H	96	10
NCP-LSH96-2	MS & MH	L	96	1 each

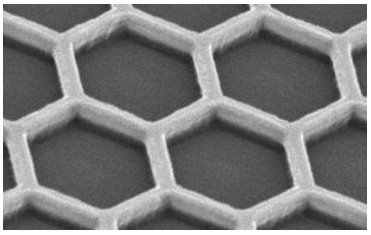
Pattern type

Pattern microsquare (MS)

Pattern microhoneycomb (MH)



Microsquare



Microhoneycomb

Attachment type

L type : Low attachment

H type : High attachment

Unpacking instruction:

Please pay **special attention** when opening the plastic packaging of the plate and avoid touching the bottom of the plate at any occasion.

The bottom of the NCP is made with a special thin and fragile film with microfabrications. Please carefully open the bag since the thin film at the bottom of the plate can be damaged even with a light pressure of the finger tip on the plate bottom.

Storage and expiration date

Plates should be stored at the dark place to avoid a direct light exposure especially UV light. Plate is sterilized before shipping. For best results, please use NCP before expiration date printed on the label.

Limitations and precautions

1. The NanoCulture® Pate is for research use only.

2. The bottom of the NCP is made with a special film with microfabrications. Please note that the structure might be damaged with pipette tips, fingers or any sharp object.

To avoid touching the bottom 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.

HANDBOOK FOR 3D CELL (SPHEROID) CULTURE

Material

・Sterile PBS(-)
・Cell dissociation reagent , for example Trypsin/EDTA
・Growth medium. Medium used for growth of the cell line you want to evaluate
・Other equipment, reagents used for conventional 2D cell culture

Experiment outline

Experiment outline provides overview of the major steps in the protocol for 3D cell culture. The detailed instructions are provided below.

- **Preparation of cell line you want to evaluate**
- **Pre-incubation of NanoCulture® Plate**
- **Preparation of cell suspension for culture on the NCP**
- **Cell culture on the NCP**

Detailed Protocol for 3D cell culture

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

Preparation of cell line 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-incubation of NanoCulture® Plate

*It is highly recommended to **pre-incubate** NCP before use. This step should be performed first and the plates can be incubated while you prepare suspension of cell line you want to evaluate on the NCP. Medium should be equilibrated to room temperature or 37℃ before use.*

3. Add the proper volume of culture medium[*1] (approximately 100 μl/well) into the wells before use. **NOTE:** If an air bubble is introduced by pipetting, we recommend taking another 100 μl of medium and pipette up and down to remove the bubble from the well.

3'. Alternatively, add half of the proper volume of culture medium[*1] (approximately 50 μl/well,) into the wells. Then centrifuge the NCP at 300-500 x g for 1-3 min to remove the bubble from the well. **NOTE:** If centrifuge machine is available, we recommend this method. Please refer

to step 9' instead of step 9 when you chose this method.
*1 If serum-free medium is used, the cell adhesion might decrease.

4. Incubate plate for 15-30 min at room temperature or 37℃ in the tissue culture hood. **NOTE:** This pre-incubation step helps to avoid air bubble during culture.

Preparation of cell suspension for plating on the NCP

5. Remove the culture medium of the maintained cells and then wash the cultured cells with PBS (-) twice.

6. Dissociate cells from the plate by adding proper cell dissociation reagent onto cells (for example Trypsin/EDTA solution) using your standard volume.

7. When cells are dissociated, add the culture medium (for example 2-3 ml) and then re-suspend the cells until you reach single cell dispersion. **NOTE:** If cells are dispersed well, uniform spheroid will be formed.

8. Count the number of cells in the suspension.

9. Adjust the cell density to 0.5 ~ 1 x 10^5 cells /ml using the culture medium. You will use 100 μl of this suspension to seed cells on the NCP (1 x 10^4 cells /well). Go to step 10. **NOTE:** The density of the cell can be adjusted to various densities depending on your experiment.

9'. (If you performed step 3') Adjust the cell density to 1 ~ 2 x 10^5 cells /ml using the culture medium. You will use 50 μl of this suspension to seed cells on the NCP (1 x 10^4 cells /well). Go to step 10'.

Cell culture on the NCP

10. Remove pre-incubated medium from the wells of NCP immediately before cell seeding. Then add 100 μl of the cell suspension you have prepared to the wells. **NOTE:** The bottom of the well should not be touched by the tip. Place the tip along the one side of the cell wall and pipette medium up or aspirate. To avoid drying the surface of the well, cell suspension should be added within 10 minutes after removal of medium.

10' (If you performed step 9') Add 50 μl of the cell suspension you have prepared to the wells.

11. Optional: 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 **(for example, NUNC cat. #236366)** is effective.

12. Place the NCP plate in the CO_2 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.

RELATED PRODUCT INTRODUCTION

Product		Cat. #
NanoCulture® Plate	MS pattern	NCP-LS96-2, NCP-LS96-10
		NCP-LS24-2, NCP-LS24-10
		NCP-HS96-2, NCP-HS96-10
		NCP-HS24-2, NCP-HS24-10
	MH pattern	NCP-LH96-2, NCP-LH96-10
		NCP-LH24-2, NCP-LH24-10
		NCP-HH96-2, NCP-HH96-10
		NCP-HH24-2, NCP-HH24-10
	MS/MH pattern	NCP-LSH96-2
		NCP-LSH24-2
NanoCulture® Medium	M type	NCM-M50
		NCM-M100
		NCM-M200
	R type	NCM-R50
		NCM-R100
		NCM-R200
Spheroid Dispersion Solution(4X)		SD4X
Spheroid Lysis Buffer		SLB

CONTACT INFORMATION

Technical and Customer Support & Manufacturer:

SCIVAX Corporation

Email: cell@scivax.com

URL: www.scivax.com/cell

Headquarters:

The east building 502, Kanagawa Science Park

3-2-1 Sakado, Takatsu-ku, Kawasaki-shi, Kanagawa 213-0012 Japan