User Guide for PAMCELL™

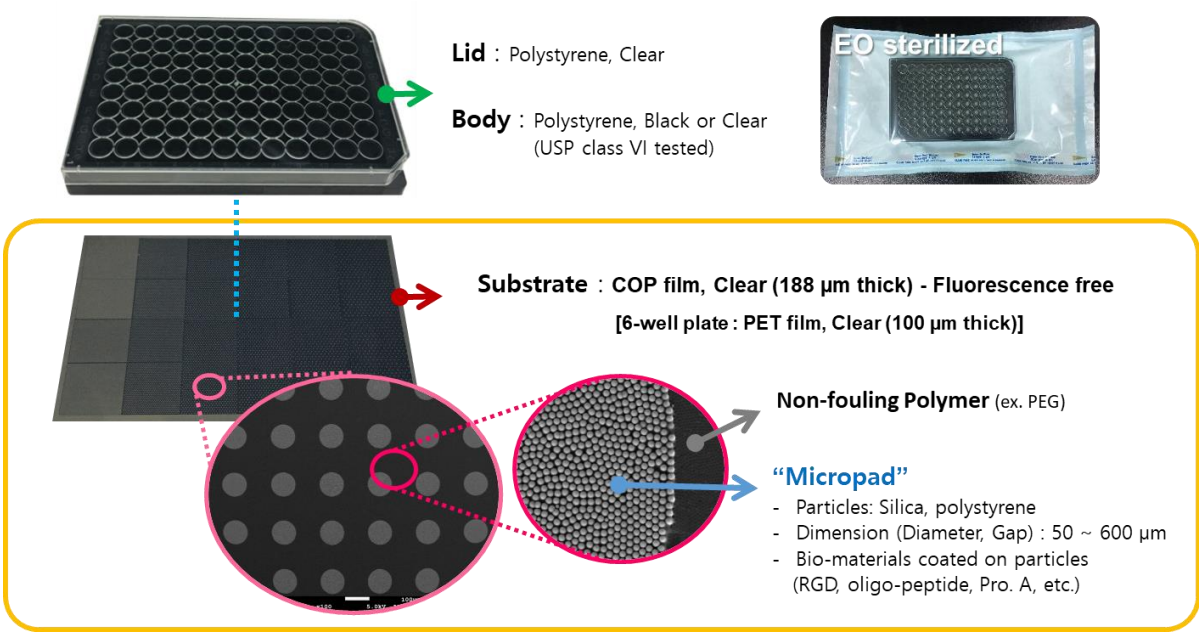
Introduction & Features

PAMCELL™ is a 3-dimensional cell culture plate which enables the formation of uniform and a wide range of sized spheroids. Plates are composed of hexagonally-arrayed spherical particles coated with cells adhesively controlled functional groups on the surface. These plates allow for the control of cell-to-substrate and cell-to-cell interactions. Plates are available in two types: R Series and T Series.

Features

- Enables formation of uniform and wide range sized spheroids.
- Optically transparent for in situ microscopic observation in automated high throughput screening systems.
- Provides various patterns on a single plate without physical barriers.

Product Information



- **Sterilization**: Ethylene oxide gas sterilization
- **Packaging material**: Nitrogen gas sealed 2-layer pouch packaging.
  [Inner layer - gas permeable film / outer - aluminum coated pouch]

PAMCELL™ plates are only for research use.

Instructions

1. PAMCELL can be used without any pretreatment, like as a common culture plate. There is no dedicated observation equipment. It is suggested to open the package in the clean bench or under sterilized environment.
2. For 96-well plate, required media is 0.1~0.3 ml for each well (recommendation: 0.3 ml), and for 6-well plate is 4~5 ml.
3. Recommended cell seeding density (per well):
   1) 96-well plate:

   [R100, R250, Test plate: 10,000~40,000 cells] / [R600: 50,000 ~ 100,000 cells]

   2) 6-well plate:

   [R100, R250, Test plate: 3~12 x 10^5 cells] / [R600: 15 ~ 30 x 10^5 cells]

   *The volume of media and cell density may need to be optimized for specific cell type.
4. Typically ideal period for exchanging media (with 70~80% volume of existing media) is 2-3 days, although it can vary for the cell type.
5. Within 24 hours, cells will be attached on the cell plate. It is recommended not to apply strong vibration or shock on the plate during initial 24 hr. Between 48~72 hr, cells spontaneously migrate to the micropads of the plate.
6. Pipetting is the best method for harvesting spheroids from the plate. It is possible to use commercially available cell scraper for harvesting, although it is not recommended.

Storage and Shelf Life

Keep the packages sealed and stored at room temperature (20~25°C) and out of direct sunlight. We recommend to use the PAMCELL plates at least 12 months from date of production. The production date is printed on the package. Once opened, use rapidly.

Trouble Shooting

Problems	Possible solutions
Relatively large difference in cell density at each micropad during initial cell adsorption period	Use a cell strainer for cell seeding.
Small number of cells on micropads	Increase cell seeding density.
Fluorescence background from the bottom plate	Use PAMCELL plate made with fluorescence free COP film as substrate.

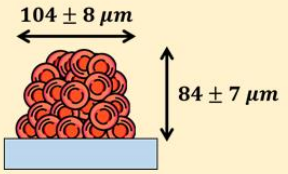
Reference Data

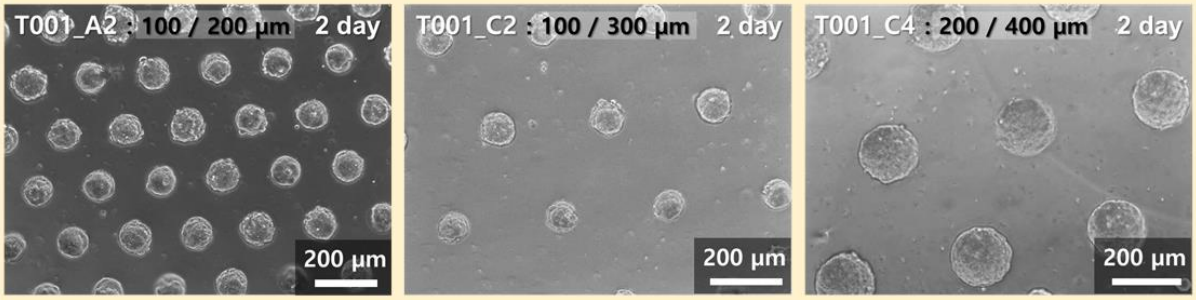
➢ Three 3D tumor cell spheroids cultured on PAMCELL

- Schematic drawings are based on spheroids cultured on (100 / 200 ㎛)* plate.

FaDu (Epidermoid)

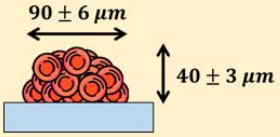
- Medium: DMEM/FBS, Cell density: 10,000 cells/well
- Spheroid shape: Bell type

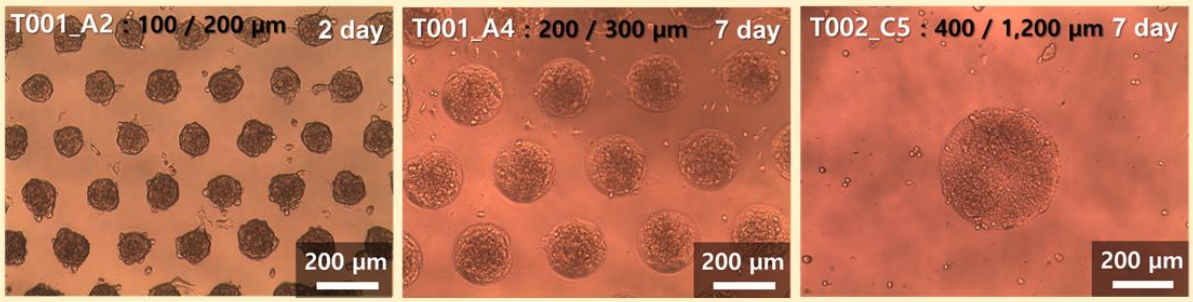




A549 (Lung carcinoma)

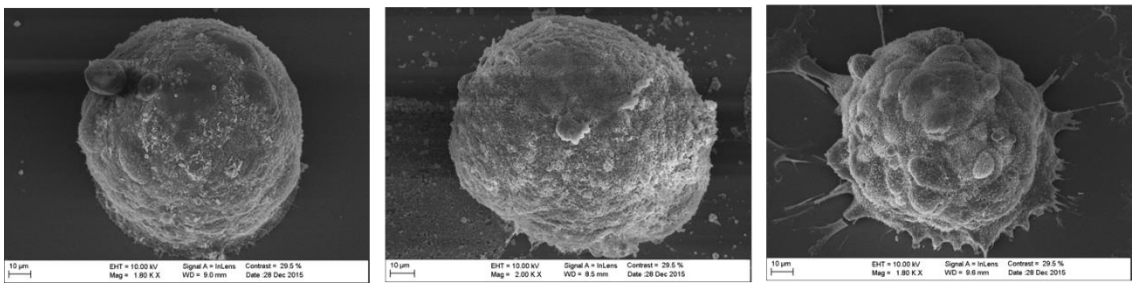
- Medium: DMEM/FBS, Cell density: 20,000 cells/well
- Spheroid shape: Bell type





*100 ㎛ micropad diameter, 200 ㎛ center-center spacing between micropads: indicates as (100/200 ㎛)

FaDu (Epidermoid) HT-29 (Adenocarcinoma) A549 (Lung carcinoma)



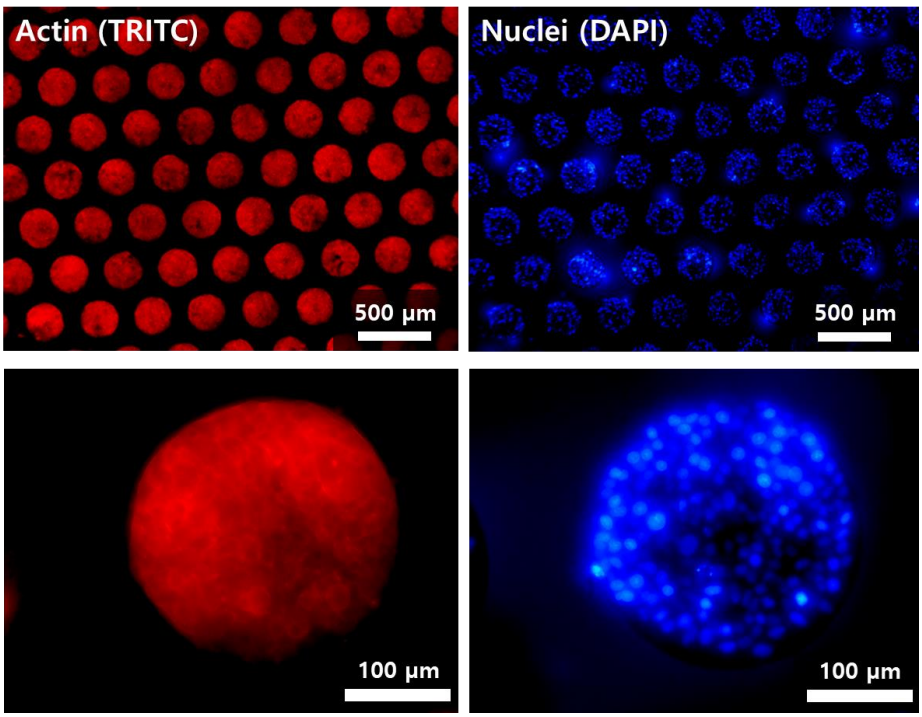
[25° tilted FE-SEM images of three tumor cells]

Cell type	Height (H, μm)			Diameter (D, μm)			H/D ratio		
	Aver.	Min.	Max	Aver.	Min.	Max	Aver.	Min.	Max
FaDu	**84**	58	126	**104**	83	154	**0.79**	0.69	0.82
HT-29	**101**	86	110	**126**	97	150	**0.81**	0.73	0.89
A549	**40**	29	75	**90**	77	126	**0.43**	0.38	0.60

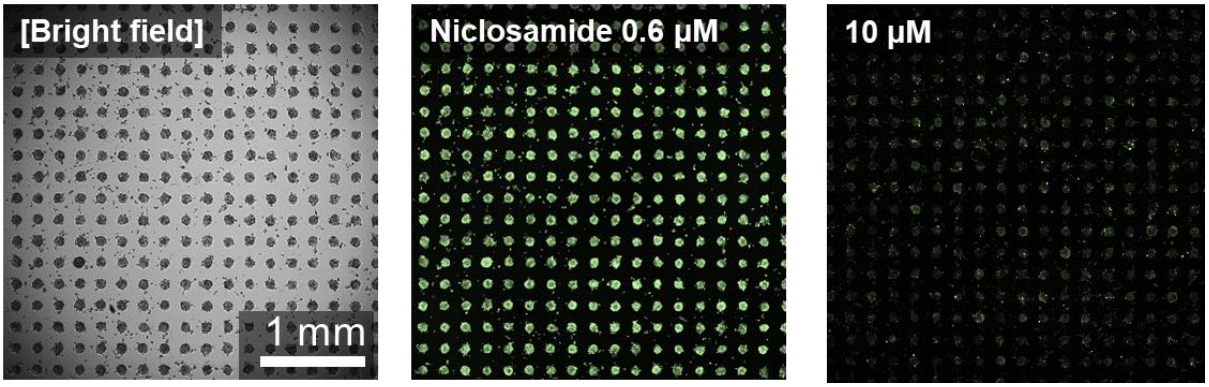
[Dimension analysis of three tumors cells from confocal microscopic images]

➢ Fluorescence microscopic images: Actin and Nuclei staining of A549 cells

- Plate: COP (Fluorescence free) / 96-well plate (250/350 μm)
- Microscopy: Olympus IX51 – 4x/0.13, 40x/0.55 Lens



- High Content screening imaging: Calcein AM staining of A549 cells (Treated with Niclosamide)
  - Plate: COP / 96-well plate (R100)
  - Instrument: Molecular Device ImageXpress Micro4



Contact Information

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