SPHEROID GROWTH, VIABILITY AND CELL PROLIFERATION

INTRODUCTION

To assess the viability and cytotoxicity of spheroids, closely related parameters that are commonly evaluated in cancer cell culture and cell cycle analysis.

3D culture of cells possessing physiological cell-cell contacts and cell-extracellular matrix contacts are known as spheroids. Due to physiological properties of 3D cell culture such as nutrient, drug, and oxygen uptake, they are often used as models of microtumours or microtissues. BIOMIMESYS® hydroscaffold facilitates consistent 3D spheroids in a standard plate format.

MATERIALS & METHODS Materials required:

- Cells grown in BIOMIMESYS® hydroscaffold e.g. HT29
- Calcein and Ethidium Bromide fluorescent dyes (e.g. Live/dead® kit from Life technologies) for viability analysis
- Antibodies for cell cycle analysis (e.g. Ki67 & Propidium Iodide)
- Fluorescence microscopy and Flow cytometry

Matrix properties:

Translucent, porous and biodegradable.

Protocols:

Culture cells in BIOMIMESYS® for the required amount of time with the corresponding changes of cell medium to achieve growth.

Seeding protocols (dependent on cell type, e.g.: 50 000 HT29 cells/well):

1. Add cells in 25-50 µL of cell culture medium (N.B. 30 µl for primary cells dependent on cell type)
2. Incubate 30 minutes as usual (37°C; 5% CO2)
3. Add medium to obtain qs. 200µl

Analysis:

1. Qualitative analysis: use the dyes in accordance with the manufacturer's instructions directly on the matrix

2. Quantitative analysis: the spheroid size can be measured accurately using suitable software.

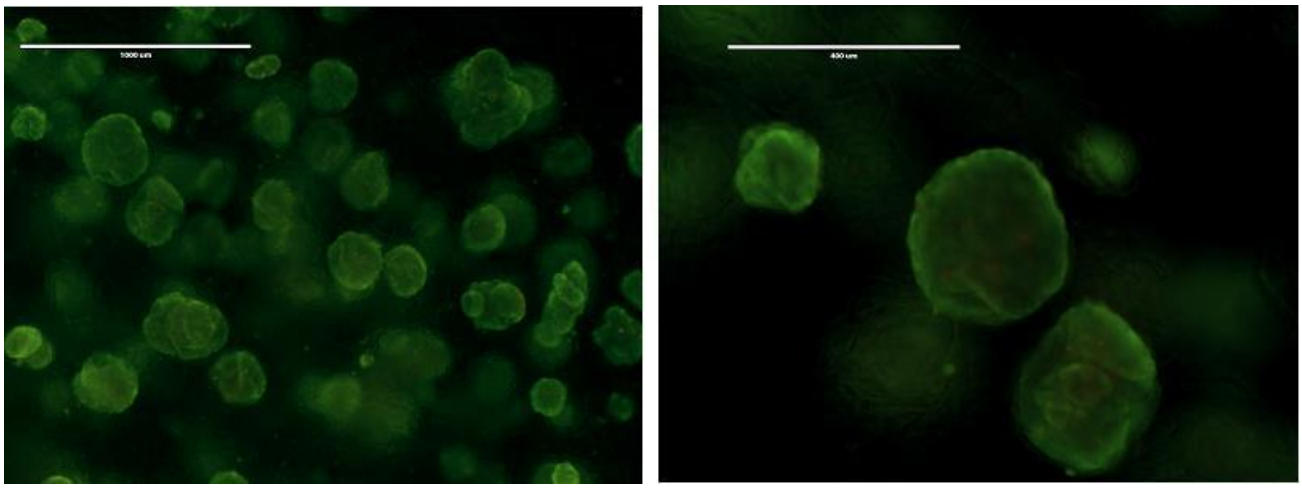
Protocol for cell retrieval:

- Hyaluronidase (bovine testes) is used to digest the hydroscaffold, the spheroids can then be recuperated.
- Detachin™ Cell Detachment Solution, a gentle enzymatic reaction, enables the spheroids to dissociate into individual cells.

92% of recuperated HT29 cells are viable.

RESULTS

Cancer cells growing in BIOMIMESYS® proliferate and aggregate to form spheroids from the 5th day of culture.

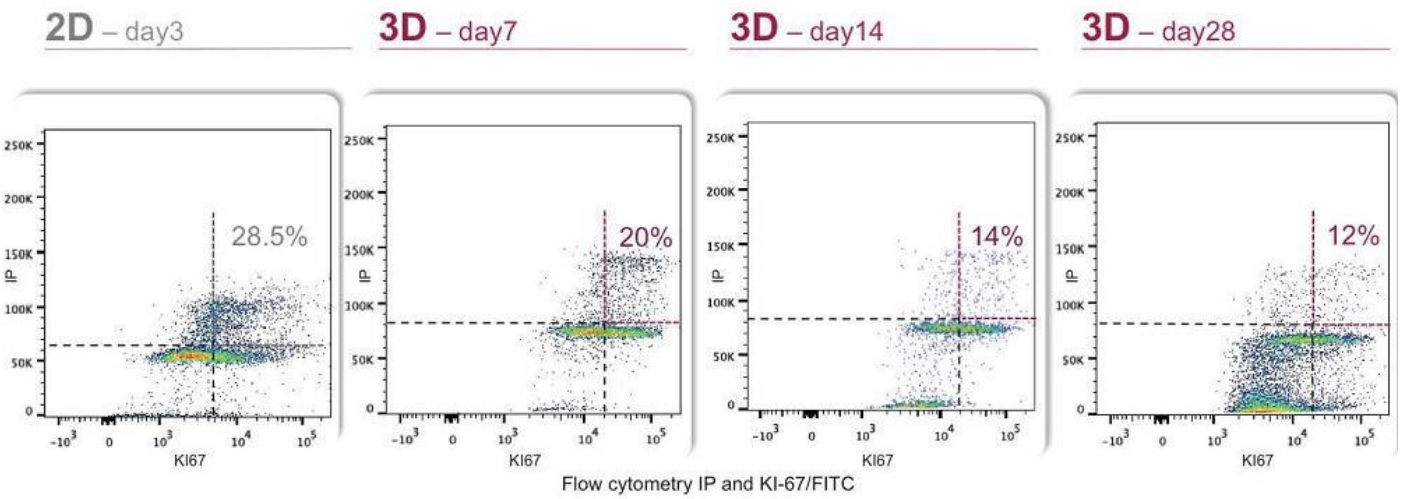


HT29 day28 live/dead® kit (Life Technologies)

The size and formation of the spheroids varies as a function of:

- **Seeding density**
- **Length of culture**
- **Cell type**

The cells can be cultured for at least 28 days. The tumor microenvironment is recreated due to the cell-cell and cell-matrix interactions BIOMIMESYS® enables.



Propidium Iodide (PI) = DNA quantity and Ki-67= cell proliferation marker

In 3D, there is less cell proliferation compared with traditional cell culture.

Proliferation diminishes over time and is correlated with a reduction in the rate of spheroid growth.

Contact Information

HCS Pharma

hello@biomimesys.com

www.biomimesys.com