cells in focus ibidi

FDA/PI Live/Dead Staining Using L929 Spheroids in the µ-Slide Spheroid Perfusion

This Application Note is an example protocol for a fluorescence staining of spheroids in the µ-Slide Spheroid Perfusion.

We are using fluorescein diacetate (FDA) and propidium iodide (PI), which is a standard staining to distinguish between viable and dead cells, respectively. FDA is taken up by living cells, which convert the non-fluorescent FDA into the green fluorescent metabolite fluorescein. The measured signal serves as an indicator for viable cells, as the conversion is esterase-dependent. In contrast, the nuclei staining dye PI cannot pass through the cell membrane of a living cell. Only in dead cells, it reaches the nucleus by passing through disordered cell membrane areas and intercalates with the DNA double helix of the cell.

The simultaneous use of these two fluorescent dyes allows for an easy two-color discrimination of both populations of living and dead cells in 3D spheroids.

Important Note: Compared to standard protocols, stainings with the µ-Slide Spheroid Perfusion need more incubation time and more washing steps. This is to ensure sufficient diffusion of dyes and antibodies into the niche area.

Related Documents:

- Application Note 63: Generation and Dynamic Culture of L929 Spheroids in the µ-Slide Spheroid Perfusion

Keywords:

- Fluorescence microscopy, live cell imaging, live/dead cell staining, fluorescein diacetate (FDA), propidium iodide (PI)

Material:

- µ-Slide Spheroid Perfusion Bioinert (80350, ibidi, Germany) prepared with L929 spheroids, see Application Note 63 for details
- PI (CN74.2, Carl Roth)
- FDA (F7378, Sigma Aldrich)
- Cell culture medium (21875034, RPMI-1640, Gibco)
- Fluorescence microscope with appropriate filter sets

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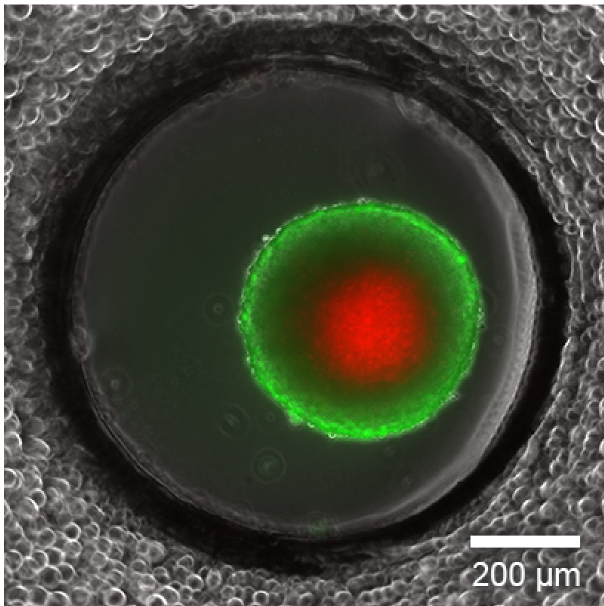
1. Staining

- Prepare the staining solution in serum-free cell culture medium to get the final concentration of PI: 200 µg/ml and FDA: 80 µg/ml.
- Perform a medium exchange according to the instructions and fill up with the staining solution.
- Incubate for 30 minutes at 37°C and 5% CO_2.
- Perform a medium exchange and fill up with cell culture medium. This washing step will remove the staining solution. Wash three times. Wait for 10 minutes between each washing step.

2. Imaging

- Observe the cells under a fluorescence microscope with appropriate filter sets.
- Optionally, overlay the images to create a merged image.

3. Results



FDA/PI live/dead staining of an L929 spheroid in the µ-Slide Spheroid Perfusion, Bioinert, after 14 days in culture with perfusion using the ibidi Pump System, 0.75 ml/min. Green: living cells (fluorescein diacetate, FDA); red: dead cells (propidium iodide, PI). Widefield fluorescence microscopy, 10x objective lens.

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