Live/dead staining with FDA and PI

1. General information

Fluorescence-based live-dead assays can be used to evaluate the viability of mammalian cells. Simultaneous use of two fluorescent dyes allows a two-color discrimination of the population of live cells from the dead-cell population. In this Application Note we present a staining protocol using fluorescein diacetate (FDA) and propidium iodide (PI), which stain viable cells and dead cells, respectively. The staining protocol is applicable to adherent cells, single cells embedded in extracellular matrix and cellular clusters, for example multicellular spheroids.

2. Principle

Live/dead staining can be performed with FDA and PI. FDA is taken up by cells which convert the non-fluorescent FDA into the green fluorescent metabolite fluorescein. The measured signal serves as indicator for viable cells, as the conversion is esterase dependent. In contrast, the nuclei staining dye PI cannot pass through a viable cell membrane. It reaches the nucleus by passing through disordered areas of dead cell membranes, and intercalates with the DNA double helix of the cell.

3. Material

For this protocol the following material and equipment is necessary:

- Fluorescein diacetate (for example: Sigma-Aldrich Co. LLC, C-7521))
    - FDA stock solution is prepared by dissolving 5 mg of FDA in 1 ml aceton (store stock solution at -20 °C)
- Propidium iodide (for example: Sigma-Aldrich Co. LLC, P4170)

  - PI stock solution is prepared by dissolving 2 mg of PI in 1 ml PBS (store stock solution at 4 °C)
- Cell culture medium
- µ-Slide 8-well, ibiTreat (ibidi, 80826)
- Inverted fluorescence microscope with filter sets for Texas Red and FITC

4. Staining protocol

Table 1 FDA/PI staining solution

Component	Volume
Culture medium without FCS	5 ml
FDA (5 mg/ml)	8 µl
PI (2 mg/ml)	50 µl

The staining solution should be freshly prepared.

Staining protocol for adherent cells and single cells embedded in extracellular matrix:

1. Preparation of the staining solution according to table 1 (keep in refrigerator).
2. Removal of medium.
3. Addition of staining solution. The amount is dependent on the geometries of the used slide or culture dish.
4. Incubate cells at room temperature for 4 to 5 minutes in the dark.
5. Removal of staining solution and addition of PBS or medium without FCS.
6. Analyze sample with fluorescent microscopy.

Staining protocol for cellular clusters:

1. Preparation of the staining solution according to table 1 (keep in refrigerator).
2. Collection of cellular clusters, if necessary add centrifugation step.
3. Removal of supernatant.
4. Addition of 1 ml staining solution.
5. Incubate sample at room temperature for 4 to 5 minutes in the dark.
6. Removal of staining solution and addition of PBS or medium without FCS.
7. Transfer of cellular clusters into a µ-Slide 8-well.
8. Analyze sample with fluorescent microscopy.

5. Examples

Example pictures of single cells:

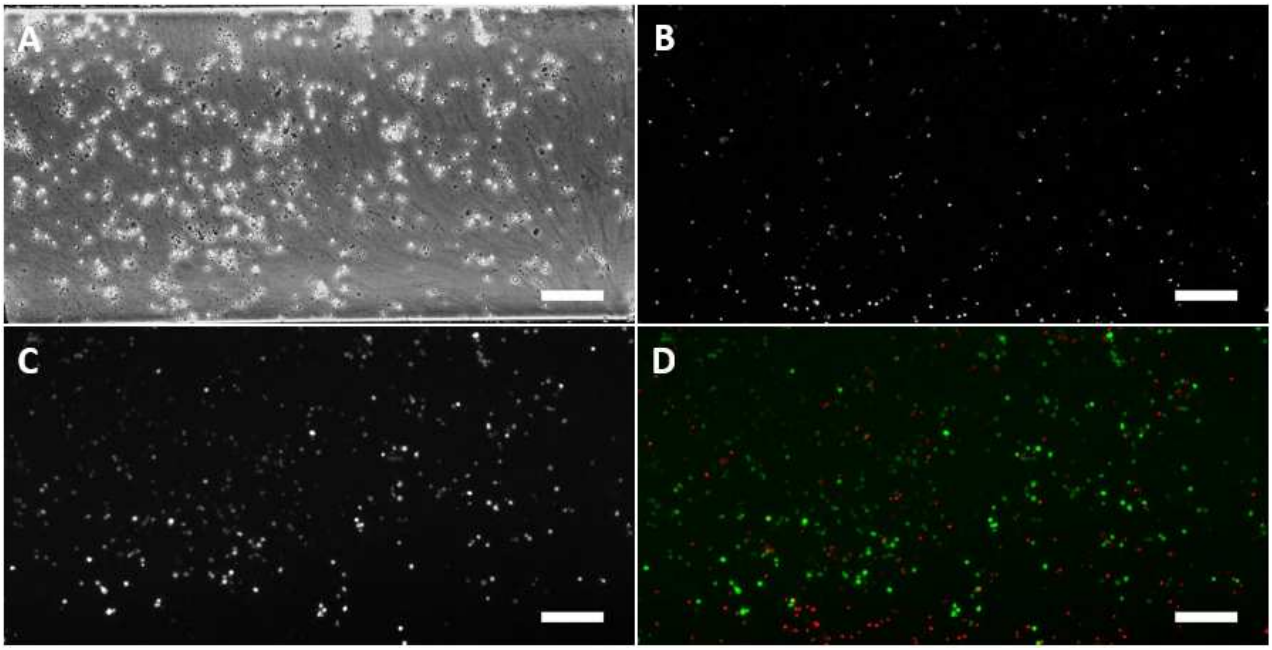

Figure 1 Fluorescence staining of Jurkat cells embedded in collagen gel revealed 65% cell viability (A: phase contrast image; B: PI-signal; C: FDA-signal; D: composite of FDA and PI signal). (Scale bar: 200 µm)

Example pictures of spheroids:

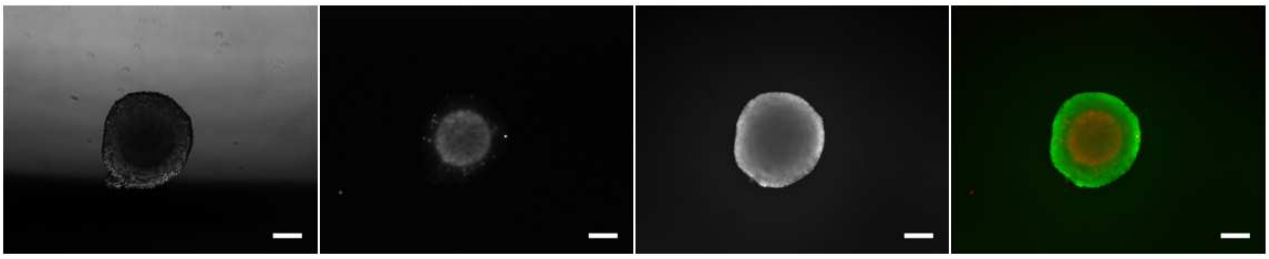

Figure 2 Vitality staining of a MCF-7 spheroid (from left to right: phase-contrast image, PI-signal, FDA-signal, composite of FDA and PI signal). (Scale bar: 200 µm)

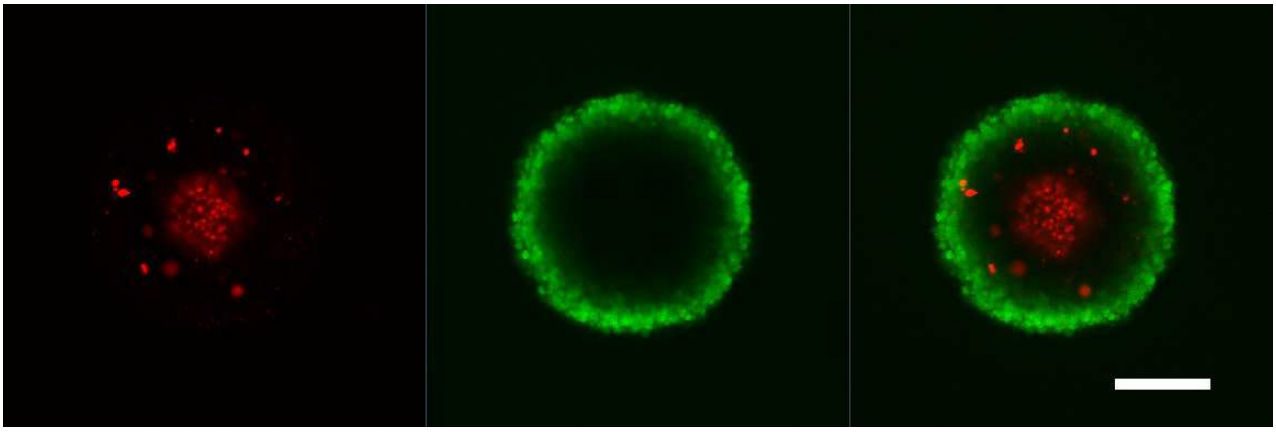

Figure 3 Confocal laser scanning microscopy images of a MCF-7 spheroid reveal the internal structure of the spheroid. (Scale bar: 200 µm)