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 living 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 3D cell 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 without FCS
- PBS or alternative buffers
- Inverted fluorescence microscope with filter sets for Texas Red and FITC

4 Staining protocol

The staining solution should always be freshly prepared and not been used for longer than 2 hours. Keep it protected from light and place it at 4°C when not needed.

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

4.1 Staining protocol for single cells

Adherent cells as well as single cells embedded in an extracellular matrix can be stained using the following protocol:

1. Preparation of the staining solution according to table 1 (keep in refrigerator).
2. Removal of cell culture medium.
3. Addition of staining solution. The volume is dependent on the geometries of the used slide or culture dish (refer to the corresponding product instructions).
4. Incubate cells at room temperature for 4 to 5 minutes in the dark.
5. Removal of staining solution.
6. Wash your sample with PBS.
7. Add PBS or medium without FCS to you sample.
8. Analyze sample with fluorescent microscopy.

4.2 Staining protocol for 3D cell cultures

The following protocol can be used for 3D cell cultures, for example multicellular spheroids or small tissue samples.

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.
7. Wash you sample with PBS.
8. Add PBS or medium without FCS to your sample.
9. Transfer of cellular clusters into a µ-Slide 8-well (ibidi, 80826).
10. Analyze sample with fluorescent microscopy.

5 Trouble shooting

- **Weak signal**
  The dye concentrations or the incubation time might have to be adopted for your condition. Furthermore, make sure that you do not use your staining solution for more than two hours. Keep the staining solution at 4°C in the dark.

- **High background signal**
  It might be possible that medium components interfere with the fluorescence signal. Make sure that serum free medium is used. Other alternatives could be the use of phenol red free medium or PBS. An additional washing step after removing the staining solution can also decrease the background signal.

- **Time dependency of the fluorescence signal**
  Bleaching effects are occurring over the time. Therefore, fast work is essential to ensure reproducible conditions. Staining only a limited number of samples at once is recommended when handling a higher number of samples. A stage top incubator is recommended to ensure optimal growth conditions for your cells.

6 Examples

6.1 Example pictures of single cells

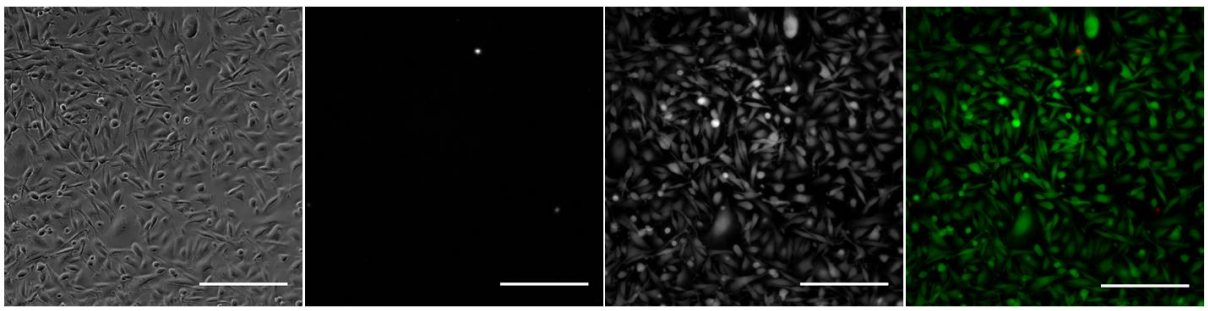

Figure 1 Vitality staining of the adherent cell line MDA-MB-231 revealed high cell viability (from left to right: phase contrast image, PI-signal, FDA-signal, composite of FDA and PI signal). (Scale bar: 200 µm)

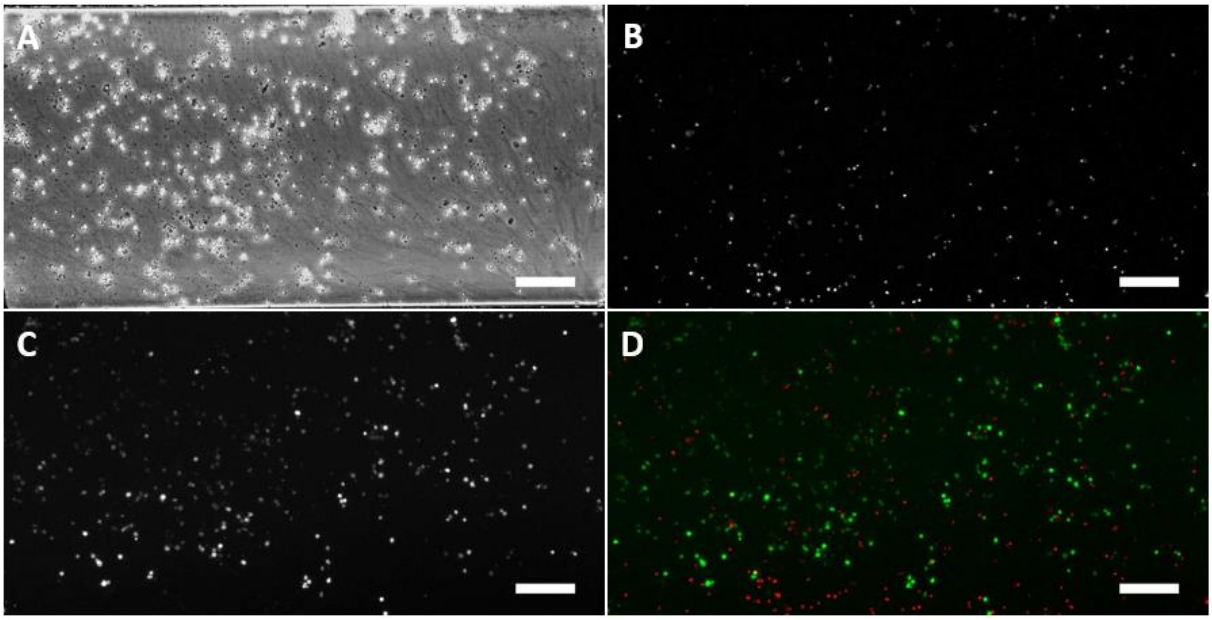

Figure 2 Vitality staining of Jurkat cells embedded in a collagen gel revealed a cell viability of 65%. (A: phase contrast image; B: PI-signal; C: FDA-signal; D: composite of FDA and PI signal). Detailed protocols for Collagen gels can be found on Application Note 26. (Scale bar: 200 µm)

6.2 Example pictures of spheroids

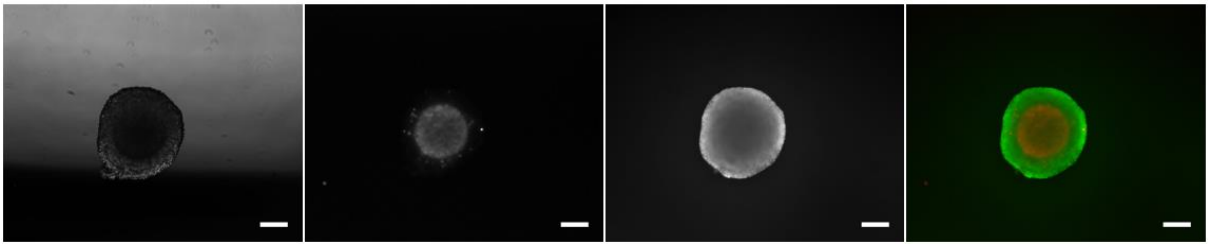

Figure 3 Vitality staining of a MCF-7 spheroid (from left to right: phase-contrast image, PI-signal, FDA-signal, composite of FDA and PI signal). Detailed information about the generation of spheroids can be found in Application Note 32. (Scale bar: 200 µm)

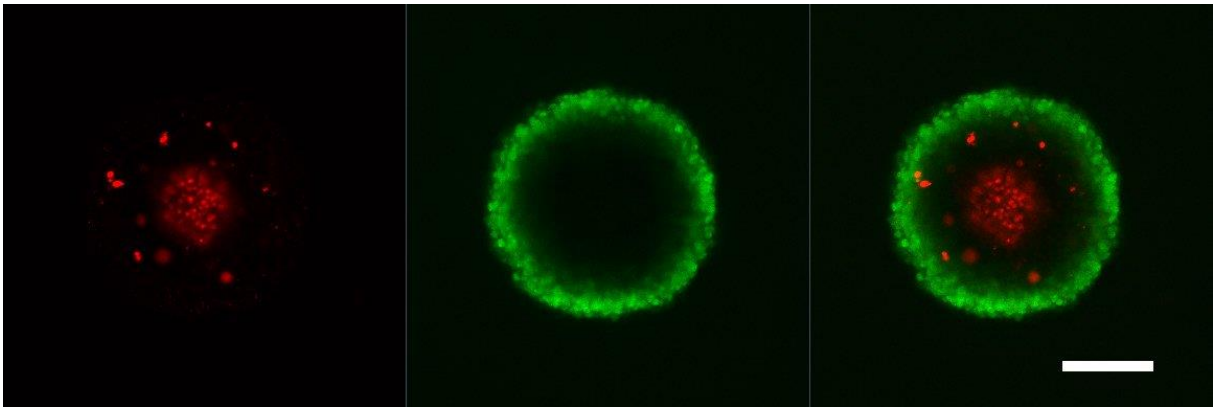

Figure 4 Confocal laser scanning microscopy images of a MCF-7 spheroid reveal the internal structure of the spheroid: a necrotic centre is surrounded by a layer of viable cells (from left to right: PI-signal, FDA-signal, composite of FDA and PI signal). (Scale bar: 200 µm)