Fluorescent viability assays on the Countess® II FL Automated Cell Counter

Introduction

Assessing cell viability is a key step in daily cell manipulation and is required for accurate and efficient downstream processing. In bright-field microscopy, the most commonly used method is trypan blue staining; however, many fluorescent options also exist and are commonly used in microscopy and flow cytometry applications.

Three commonly used viability kits are discussed within this application note, but with the flexibility offered by EVOS® Light Cubes, one has the ability to analyze cell viability using a variety of proven reagents, including: propidium iodide, ethidium homodimer, SYTOX® dyes, 7-AAD, ReadyProbes® reagents, acridine orange, and kits such as the LIVE/DEAD® Viability/Cytotoxicity Kit.

General Methods

Materials

- Countess® II FL Automated Cell Counter (Cat. No. AMQAF1000)
- Countess® Cell Counting Chamber Slides (Cat. No. C10228) or Countess® II FL Reusable Slide (Cat. No. A25750)
- Cell viability dyes; commonly used kits include:
  - LIVE/DEAD® Viability/Cytotoxicity Kit (Cat. No. L3224)—used for live and dead cell confirmation
  - ReadyProbes® Cell Viability Imaging Kit, Blue/Green (Cat. No. R37609)—total cell stain plus dead cell stain
  - ReadyProbes® Cell Viability Imaging Kit, Blue/Red (Cat. No. R37610)—total cell stain plus dead cell stain

Instrument setup

1. Turn on the Countess® II FL Automated Cell Counter and install the appropriate EVOS® Light Cubes.
2. Install the appropriate slide holder for either the disposable or reusable slide.
3. Obtain a Countess® disposable or reusable slide.

Protocol

1. Acquire a eukaryotic cell suspension and the desired cell viability reagent.
2. Stain the cell sample according to the instructions for the viability reagent.
3. Apply 10 µL of the stained sample to the Countess® slide.
4. Insert the slide into the Countess® II FL instrument's sample port to initiate autofocus.
5. Adjust light intensities to minimize fluorescent background.
6. Press "Count".

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Cell viability assay using the LIVE/DEAD® Viability/Cytotoxicity Kit

Ubiquitous intracellular esterase activity and an intact plasma membrane are distinguishing characteristics of live cells. The LIVE/DEAD® Viability/Cytotoxicity Kit quickly discriminates live from dead cells by simultaneously staining with green-fluorescent calcein AM to indicate intracellular esterase activity and red-fluorescent ethidium homodimer-1 (EthD-1) to indicate loss of plasma membrane integrity (Figure 1). It is adaptable to most eukaryotic cells where cytotoxic conditions produce these cellular effects. This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, ^{51}Cr release assay, and similar methods for determining cell viability and cytotoxicity.

Calcein AM (Ex/Em: 494/517 nm): Cell-permeant dye that is converted to fluorescent calcein by live-cell esterase activity to produce intense, uniform green fluorescence. Calcein AM is detected with standard FITC/GFP filter sets.

Ethidium homodimer-1 (EthD-1) (Ex/Em: 528/617 nm): High-affinity stain that enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, producing bright red fluorescence in dead cells. EthD-1 is excluded by the intact plasma membrane of live cells. EthD-1 can be detected with RFP and Texas Red® filter sets.

Background fluorescence levels is low with this assay because the dyes are virtually nonfluorescent before interacting with cells.

Protocol

1. Allow calcein AM (Component A) and ethidium homodimer-1 (Component B) to thaw and fully equilibrate to room temperature.
2. On the day of the viability assay, create a 2 mL cell staining solution containing 2 µM calcein AM and 4 µM EthD-1 by adding 2 µL each of Component A and Component B to ~1.99 mL of buffer. Mix thoroughly.
3. Remove the culture medium from the cells.
4. Add 100–200 µL of the staining solution directly to the cells.
5. Incubate 30 minutes at 20–25°C.
6. Apply 10 µL of the stained sample to the Countess® slide.
7. Insert the slide into the Countess® II FL instrument's sample port to initiate autofocus.
8. Adjust light intensities to minimize fluorescent background.
9. Press "Count".

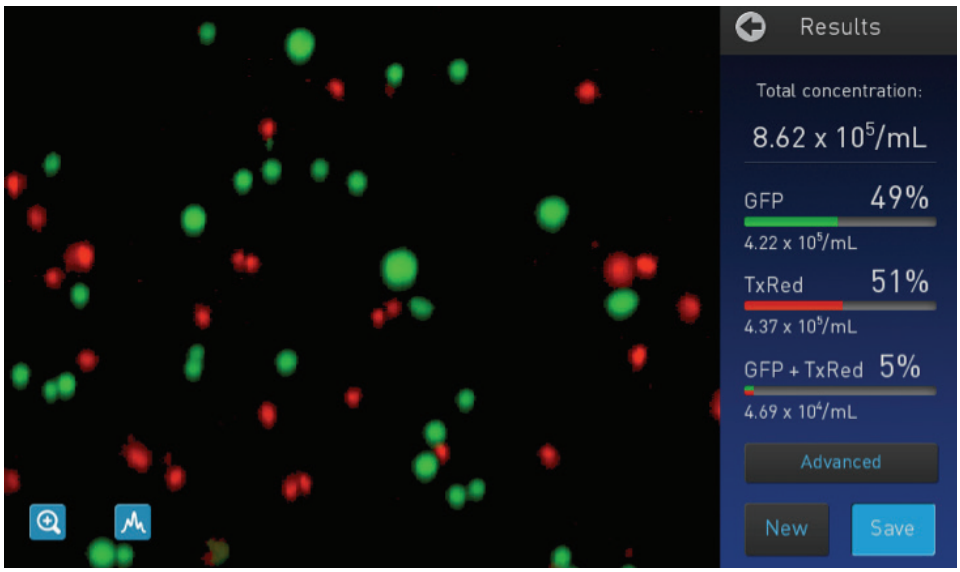

Figure 1. Viability assay via cell staining using the LIVE/DEAD® Viability/Cytotoxicity Kit. Live and heat-killed U2OS cells were mixed in an approximate 1:1 ratio, and then stained with calcein AM and EthD-1 supplied with the kit. The resultant suspension was then evaluated using the GFP and Texas Red® (TxRed) Light Cubes with the Countess® II FL Automated Cell Counter.

Cell viability assay using the ReadyProbes® Cell Viability Imaging Kit, Blue/Green

The ReadyProbes® Cell Viability Imaging Kit, Blue/Green, is a ready-to-use kit that can be used to quickly and easily determine cell viability. Just add 2 drops each of room temperature–stable NucBlue® Live reagent and NucGreen® Dead reagent to 1 mL of cell growth medium, and then determine viability by counting total vs. dead cells (Figure 2).

NucBlue® Live reagent (Ex/Em: 360/460 nm): Stains the nuclei of all cells; detected with a standard DAPI filter.

NucGreen® Dead reagent (Ex/Em: 504/523 nm): Stains only the nuclei of dead cells with compromised plasma membranes; detected with standard FITC/GFP (green) filter sets.

Suggestions for use

- NucBlue® Live and NucGreen® Dead reagents can be added directly to cells in complete growth medium or a compatible buffer solution.
- In most cases, 2 drops per milliliter of suspension and an incubation time of 5–30 minutes will give bright nuclear staining; however, optimization may be needed for some cell types, conditions, and applications. In such cases, simply add more or fewer drops to obtain the optimal staining intensity.

Protocol

1. Culture cells in an appropriate medium.
2. Add 2 drops each of NucBlue® Live and NucGreen® Dead reagents per milliliter of cell suspension.
3. Incubate 5–30 minutes.
4. Apply 10 µL of the stained sample to the Countess® slide.
5. Insert the slide into the Countess® II FL instrument's sample port to initiate autofocus.
6. Adjust light intensities to minimize fluorescent background.
7. Press "Count".

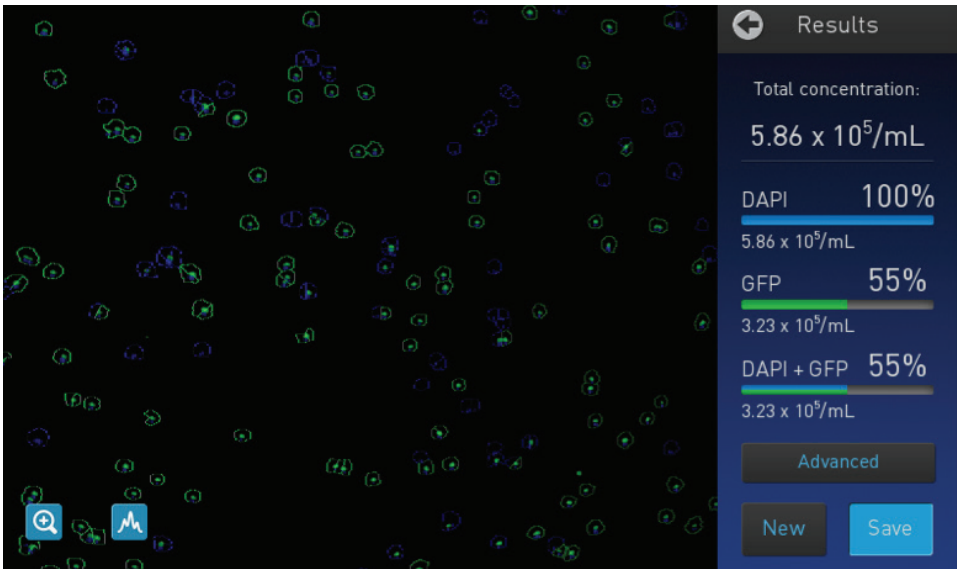

Figure 2. Viability assay via cell staining using the ReadyProbes® Cell Viability Imaging Kit, Blue/Green. Live and heat-killed U2OS cells were mixed in an approximate 1:1 ratio, and then stained with NucBlue® Live reagent and NucGreen® Dead reagent supplied with the kit. The resultant sample was then evaluated using the Countess® II FL Automated Cell Counter with DAPI and GFP Light Cubes installed.

Cell viability assay using the ReadyProbes® Cell Viability Imaging Kit, Blue/Red

ReadyProbes® Cell Viability Imaging Kit, Blue/Red, is a ready-to-use kit that can be used to quickly and easily determine cell viability. Just add 2 drops each of room temperature–stable NucBlue® Live reagent and propidium iodide to 1 mL of cell growth medium, and then determine viability by counting total vs. dead cells (Figure 3).

NucBlue® Live reagent (Ex/Em: 360/460 nm): Stains the nuclei of all cells; detected with a standard DAPI filter.

Propidium iodide (Ex/Em: 535/617 nm): Stains only the nuclei of dead cells with compromised plasma membranes; detected with standard TRITC/RFP (orange) filter sets.

Suggestions for use

- NucBlue® Live reagent and propidium iodide can be added directly to cells in complete growth medium or a compatible buffer solution.
- In most cases, 2 drops per milliliter of suspension and an incubation time of 5–30 minutes will give bright nuclear staining; however, optimization may be needed for some cell types, conditions, and applications. In such cases, simply add more or fewer drops to obtain optimal staining intensity.

Protocol

1. Culture cells in an appropriate medium.
2. Add 2 drops each of NucBlue® Live reagent and propidium iodide per milliliter of cell suspension.
3. Incubate 5–30 minutes.
4. Apply 10 µL of the stained sample to the Countess® slide.
5. Insert the slide into the Countess® II FL instrument's sample port to initiate autofocus.
6. Adjust light intensities to minimize fluorescent background.
7. Press "Count".

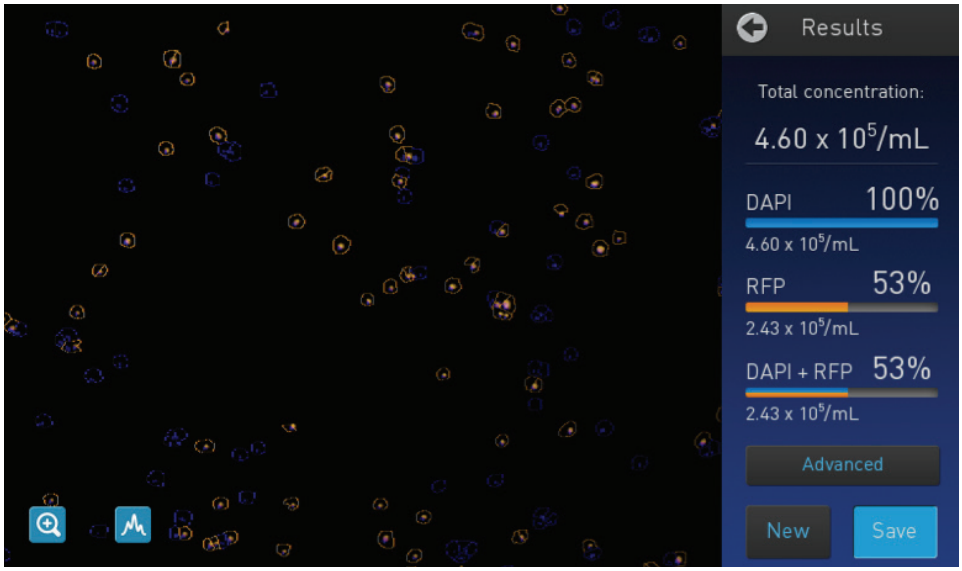

Figure 3. Viability assay via cell staining using the ReadyProbes® Cell Viability Imaging Kit, Blue/Red. Live and heat-killed U2OS cells were mixed in an approximate 1:1 ratio, and then stained with NucBlue® Live reagent and propidium iodide supplied with the kit. The resultant sample was then evaluated using the Countess® II FL Automated Cell Counter with DAPI and RFP Light Cubes installed.

Find out more at **lifetechnologies.com/countessII**

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