Fluorescent apoptosis evaluation using the Countess II FL Automated Cell Counter

Introduction

The ability to assess cell health in a cell population is a basic and critical evaluation parameter in many cell and molecular biology labs. To this end, knowing how many cells are dead or dying are key pieces of information. To answer such basic questions, the cells of interest can be stained with a viability dye, such as Invitrogen™ Molecular Probes™ SYTOX™ Red Dead Cell Stain, as well as an apoptosis indicator that measures caspase activation, such as Invitrogen™ Molecular Probes™ CellEvent™ Caspase-3/7 Green Detection Reagent. This staining combination, used together with an Invitrogen™ Countess™ II FL Automated Cell Counter equipped with GFP and Cy®5 EVOS™ Light Cubes, allows a quick and simple method to obtain apoptosis data together with cell viability data.

General methods

Materials

- Countess II FL Automated Cell Counter (Cat. No. AMQAF1000)
- Invitrogen™ Countess™ Cell Counting Chamber Slides (Cat. No. C10228) or Invitrogen™ Countess™ II FL Reusable Slide (Cat. No. A25750)
- EVOS Light Cubes, GFP (Cat. No. AMEP4651) and Cy5 (Cat. No. AMEP4656)
- CellEvent Caspase-3/7 Green Detection Reagent (Cat. No. C10423)
- SYTOX Red Dead Cell Stain (Cat. No. S34859)

Protocol—instrument setup

1. Turn on the Countess II FL Automated Cell Counter and install the GFP and Cy5 EVOS Light cubes.
2. Install the appropriate slide holder for either the disposable or reusable slide.
3. Obtain a disposable or reusable Countess slide.

Protocol—culture setup and counting

1. Acquire a eukaryotic cell suspension, CellEvent Caspase-3/7 Green Detection Reagent, and SYTOX Red Dead Cell Stain.
2. Stain the cell sample according to the manual provided with the CellEvent Caspase 3/7 Green Detection Reagent.
3. Stain the cell sample according to the manual provided with the SYTOX Red Dead Cell Stain.
4. Apply 10 µL of the stained cell sample to the Countess slide.

5. Insert the Countess slide into the Countess II FL Automated Cell Counter sample port to initiate autofocus.
6. Adjust fluorescence light intensities to minimize background while maximizing signal.
7. Press Capture.

Apoptosis assessment

The CellEvent Caspase-3/7 Green Detection Reagent (Ex/Em: 502/530 nm when bound to DNA) is intrinsically nonfluorescent, as the DEVD peptide inhibits the ability of the dye to bind to DNA. However, after activation of caspase-3/7 in apoptotic cells, the DEVD peptide is cleaved, enabling the dye to bind to DNA and produce a bright, fluorogenic response. The fluorescence emission of the dye when bound to DNA can be observed using a standard "FITC" or GFP filter set.

To use CellEvent Caspase 3/7 Green Detection Reagent, simply add substrate to control and treated cells, incubate 30 minutes, and visualize. Apoptotic cells with activated caspase-3/7 will have bright green nuclei, while cells without activated caspase 3/7 will have minimal fluorescence signal.

SYTOX dead cell stains do not cross intact cell membranes, and they exhibit increased fluorescence upon dsDNA binding, making them some of our brightest dead cell stains. SYTOX dead cell stains can be applied to cells and visualized without an additional wash step because they are nonfluorescent in aqueous media. These stains are available in multiple single-color formats, making them compatible with many filter sets.

After brief incubation with SYTOX Red Dead Cell Stain, the nucleic acids of dead cells fluoresce bright red when excited and detected with traditional Cy5 filter sets. SYTOX Red Dead Cell Stain is distinct from dead cell probes such as 7-AAD and propidium iodide (PI) that are excited using 488 nm light. Moreover, the emission of SYTOX Red Dead Cell Stain is limited to one channel, thus minimizing spectral overlap with CellEvent Caspase 3/7 Green Detection Reagent and simplifying workflow.

What used to take more than an hour on traditional microscopes or cytometers—simply acquiring preliminary results—now takes only a few minutes with the Countess II FL Automated Cell Counter. You can save time and effort by checking your sample before using a microscope or flow cytometer.

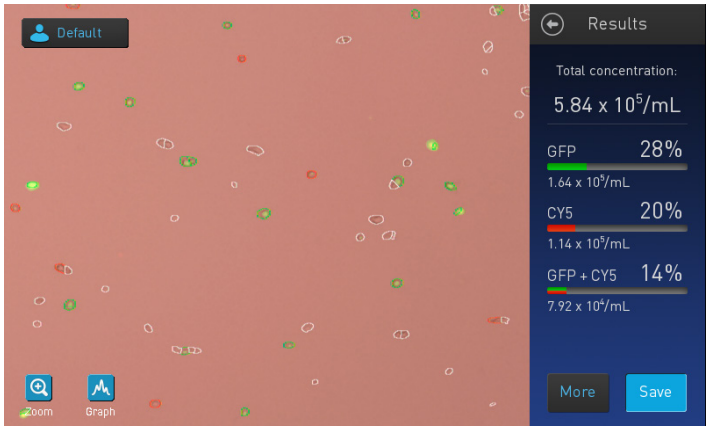

Figure 1. Apoptotic and dead cells counted on a Countess II FL Automated Cell Counter. A Countess II FL Automated Cell Counter was loaded with EVOS Light Cubes for GFP (Cat. No. AMEP4651) and Cy5 (Cat. No. AMEP4656). After incubation with 0.5 µm staurosporine, HeLa cells were labeled with 1:400 CellEvent Caspase-3/7 Green Detection Reagent (Cat. No. C10423) to identify apoptotic cells, and then stained with 1:1,000 SYTOX Red Dead Cell Stain (Cat. No. S34859) to denote all dead cells and incubated at room temperature for 30 minutes.

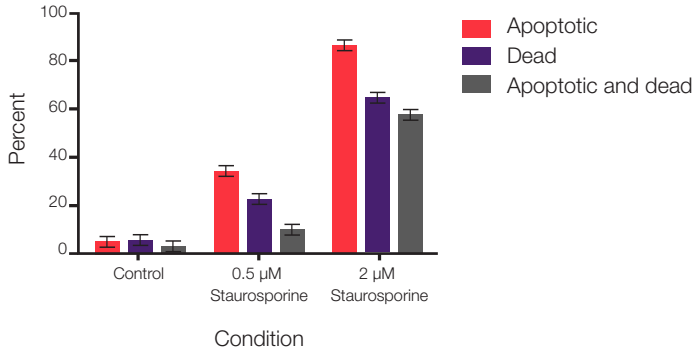

Figure 2. Apoptosis of HeLa cells measured on a Countess II FL Automated Cell Counter using CellEvent Caspase-3/7 Green Detection Reagent and SYTOX Red Dead Cell Stain. The indicated amounts of staurosporine were applied to HeLa cells to trigger apoptosis. Increases in green and far-red signal were noted with increased drug treatment, indicating significant increases in apoptosis and cell death, respectively.

Find out more at **thermofisher.com/countess**

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