APPLICATION NOTE

Countess II FL Automated Cell Counter

Fluorescent protein reporter gene transduction efficiency measured with the Invitrogen Countess II FL Automated Cell Counter

Introduction

The assessment of how many cells have been successfully transfected or transduced in a cell population is a basic and critical evaluation parameter in many cell and molecular biology labs. Commonly, the cells of interest are transfected or transduced with a construct that results in the expression of a fl uorescent protein (FP) reporter, such as GFP. The Invitrogen™ Countess™ II FL Automated Cell Counter loaded with your choice of Invitrogen™ EVOS™ light cubes allows a quick and simple method to easily obtain transfection or transduction effi ciency data. The procedures described below can be applied to any commonly used FP reporter system that is currently evaluated using microscopy or flow cytometry

Materials

- Invitrogen™ Countess™ Cell Counting Chamber Slides (Cat. No. 10399053) or Countess™ II FL Reusable Slides (Cat. No. 15311986)
- Appropriate EVOS light cube(s)
- Fluorescent protein (FP)-expressing cells

Protocol—instrument setup

1. Turn on the Countess II FL Automated Cell Counter and install appropriate EVOS light cube(s).
2. Install the appropriate slide holder for either the disposable or reusable slide.
3. Locate the slide(s) to be used.

Protocol—culture setup

1. Acquire a eukaryotic cell suspension for FP expression analysis.
2. If cell viability information is desired in addition to FP expression analysis, combine 10µL of the sample with 10µL of trypan blue. (Note: Trypan blue may quench the fluorescent signal, depending on the FP's localization.)
3. Apply 10µL of the stained sample to the counting slide.
4. Insert the counting slide into the Countess II counter's sample port to initiate autofocus, then press "Capture". Record the viability.

5. Once brightfi eld counting is complete, reinsert the sample slide and select the appropriate light source from the upper-right corner of the screen.

6. Adjust the fluorescence excitation light intensities to minimize background.

7. Press “Capture” once more.

Using the Countess II FL Automated Cell Counter and appropriate light cubes, what used to require multiple manual cell counts per sample with a hemocytometer can now be obtained in as little as 10 seconds with a single count on the Countess II FL Automated Cell Counter, while reducing the count-to-count variability between lab users. This results in better consistency between experiments and dramatic time savings. Figures 1 and 2 present data from common cell counting applications involving the analysis of transduction efficiency.

Figure 1. Determination of transduction efficiency using the Countess II FL Automated Cell Counter. A Countess II FL Automated Cell Counter was equipped with EVOS light cubes for DAPI (Cat. No. 16815411) and GFP (Cat. No. 16825411). HeLa cells were transduced with Invitrogen™ CellLight™ Mitochondria-GFP, BacMam 2.0 (Cat. No. 10243019), and then stained with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. 12303553) for total cell staining. In this case, 72% transduction efficiency was determined for the cell sample in less than 10 seconds.

Summary

Flow cytometry and manual cell counting with a hemocytometer and microscope are the gold standards for fluorescent protein expression analysis, but both require advanced user training and relatively expensive instrumentation. The Countess II FL Automated Cell Counter is able to provide comparable data, but with faster turnaround and greater cost-effectiveness while reducing user-to-user variability.

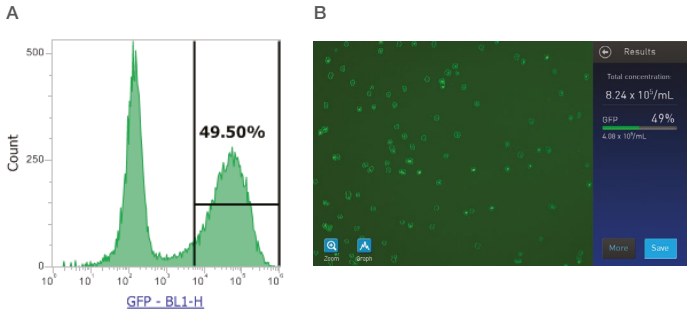

Figure 2. Fluorescent cell counts similar to those obtained by flow cytometry can be easily acquired with the Countess II FL Automated Cell Counter, with a single light cube. U2OS cells were transduced using Invitrogen™ CellLight™ Nucleus-GFP, BacMam 2.0 (Cat. No. 10292779), and allowed to incubate for 36 hours. The cells were evaluated for

fluorescent protein expression by (A) flow cytometry and (B) the Countess II FL Automated Cell Counter. The transduction percentages determined by the two methods were very close to each other.

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