Technical Note 223

Counting SF9 Cells

Introduction

Insect cells such as the SF9 cell line isolated from *Spodoptera frugiperda* are commonly used in the process of recombinant protein production using baculovirus. This method of protein production requires accurate knowledge of SF9 cell counts and viability to qualify successful transfection and healthy cells producing protein.

This technical note describes how the CellDrop FL Automated Cell Counter enables rapid and accurate cell counts and cell viability determination. Two methods were used to measure cell viability: Trypan Blue dye exclusion, and Acridine Orange / Propidium Iodide fluorescent dye labeling.

Materials and Methods

SF9 cells were gently sloughed from culture to maintain viability. Cells were centrifuged at 100 g then resuspended in PBS for counting.

Trypan Blue

Cells in PBS were mixed 1:1 with Trypan Blue (DeNovix CAT #CD-TRY-1.5) and counted using the Trypan Blue app on the CellDrop Cell Counter using the default protocol described below:

Table 1: SF9 counting protocol for the Trypan Blue app.

Dilution Factor	2
Minimum Diameter	6
Maximum Diameter	30
Live Roundness	50
Dead Roundness	25
Stained Threshold	35
Small Cell Mode	OFF
Irregular Cell Mode	OFF

AO/PI

SF9 cells in PBS were mixed 1:1 with AO/PI (DeNovix CAT #CD-AO-PI-1.5) and counted using the AO/PI app on the CellDrop FL Automated Cell Counter using the protocol outlined below:

Table 2: SF9 counting protocol for the AO/PI app.

Dilution Factor	2
Minimum Diameter	8
Maximum Diameter	20
Live Roundness	1
Dead Roundness	1
Green FL Threshold	1
Red FL Threshold	12
Irregular Cell Mode	OFF

Results and Summary

The CellDrop Automated Cell Counter counts cells and reports viability, circling live cells in green and dead cells in red. The Trypan Blue app identifies stained (dark) cells as dead and unstained cells as live and labels them appropriately (Figure 1). The AO/PI app identifies green-fluorescing cells as viable live cells, and red-fluorescing cells as dead (Figure 2). CellDrop enables rapid and accurate counting and identification of live and dead cells in a variety of mammalian and other cell types, including the SF9 insect cell line.
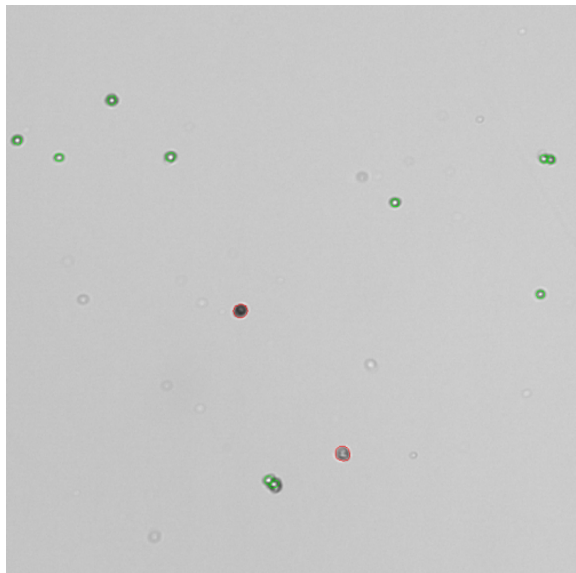

Figure 1. SF9 cells counted in the Trypan Blue app on the CellDrop Automated Cell Counter. Live cells are circled in green and dead, stained cells are circled in red.
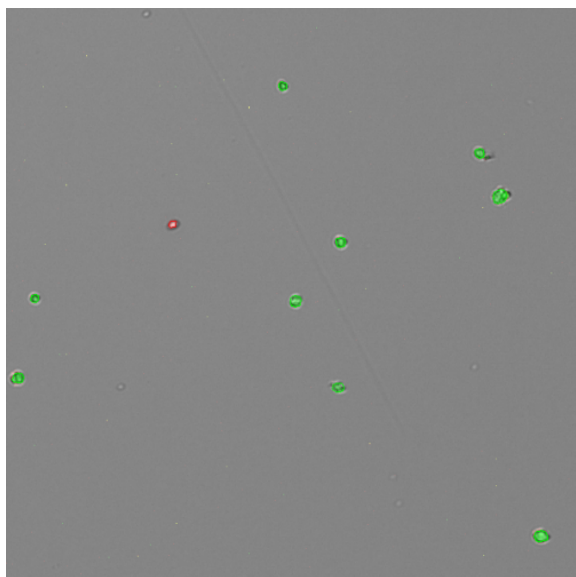

Figure 2. SF9 cells counted in the AO/PI app on the CellDrop FL Automated Cell Counter. Green-fluorescing live cells are circled in green and red-fluorescing dead cells are circled in red.

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