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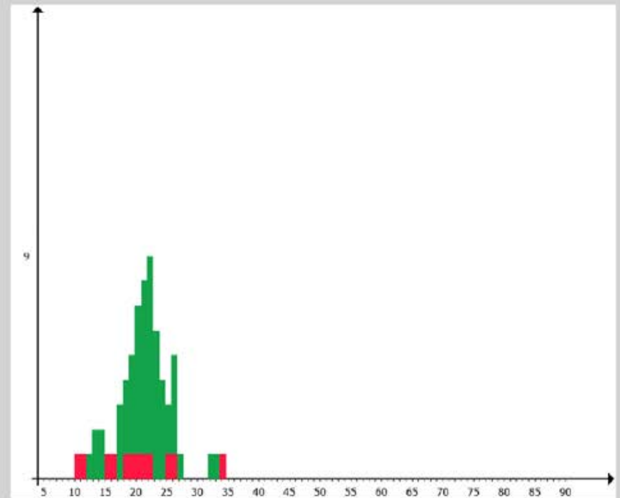

High throughput cell swelling analysis with the Spark™ 10M multimode microplate reader

Determination of cell viability and size distribution using insect cells after viral infection

Introduction

Cell counting, viability analysis and determination of size distribution are routinely performed by researchers across multiple disciplines, from standard cell passaging for downstream experiments to using insect cells infected with baculovirus to produce recombinant proteins [1], or determining the differentiation capacity of stem cells.

Tecan's Spark 10M has an integrated, bright field cell imaging module. This patent-pending system enables label-free cell counting, size distribution determination and trypan blue-based cell viability analysis with an easy-to-use, disposable Cell Chip™. Each Cell Chip has two sample chambers, and four Cell Chips can be loaded into the Cell Chip adapter (Figure 1), allowing up to eight samples to be analyzed in a single measurement run taking less than 1 minute and 45 seconds. The pre-set one click applications in the instrument's SparkControl™ software simplify reliable cell counting and viability analysis in the user's everyday workflow.

In this study, the analysis of cell concentration, cell viability and size distribution for the determination of the optimal protein production rate in recombinant Sf21 cell cultures is presented.

Figure 1: The Cell Chip adapter – four chips (eight samples) can be loaded and analyzed in one measurement run.

Material and methods

- Spark 10M multimode microplate reader equipped with Cell Chips and Cell Chip adapter (Tecan, Austria)
- Sf21 cells (ATCC obtained from LGC Standards, Germany) grown in Sf900™ III SFM medium containing 1X penicillin-streptomycin (Life Technologies, USA) at 37°C in 50 mm Unitron shakers (Infors, Bottmingen, Switzerland).
- 0.4 % trypan blue solution (Life Technologies, USA)
- Bac-to-Bac® Baculovirus Expression System (Life Technologies, USA)

Insect cells were used to produce recombinant proteins. The specific gene coding for the protein of interest was introduced into the cells using a recombinant baculovirus. Generation of the virus construct was performed according to the protocol of the Bac-to-Bac Baculovirus Expression System. Briefly, cell suspensions at 1 x 10^6 cells/ml were infected with a virus stock, typically at a ratio of 1:100 v/v. Viability and cell size was subsequently recorded every 24 hours until 72 hours after infection.

Using trypan blue to measure cell viability

One of the earliest and most common methods for measuring cell viability is the trypan blue exclusion assay. Trypan blue is cell membrane impermeable, and therefore only enters cells with compromised membranes. Within the compromised cells, trypan blue binds to intracellular proteins, rendering the cells a bluish color. The assay allows direct identification and counting of live (unstained) and dead (blue) cells in a given population (Figure 2). The Spark 10M reader offers straightforward automation of the trypan blue exclusion assay, with one click cell counting and cell viability analysis.

The SparkControl software uses an outline algorithm that reproduces the cell membrane outline around each cell. When the counting is complete, a green outline is produced for each living cell, and a red outline for each dead cell. This sophisticated algorithm allows consistent identification of the desired cell population, and provides reliable and accurate cell numbers, concentrations, viability analysis and size measurements. Most cell types can be analyzed using the system's default settings, which can be easily modified by the user to ensure accurate results. The software can also be used to count the number of cells which fall within a user-defined size range and, for this study, only cells that were between 9 and 50 µm in diameter were counted.

Cell counting procedure:

1. Insect cell culture mixed with 0.4 % trypan blue solution (1:1 v/v).
2. 10 µl of suspension pipetted into each sample chamber of the Cell Chips.
3. Cell Chips placed into the Cell Chip adapter.
4. Loaded Cell Chip adapter inserted into the Spark 10M reader.
5. One click cell viability application run in SparkControl to obtain cell concentration, viability and cell size diameter.

Results

We have used one of the most common insect cell lines, Sf21 in order to verify the protein production level 72 hrs post infection.

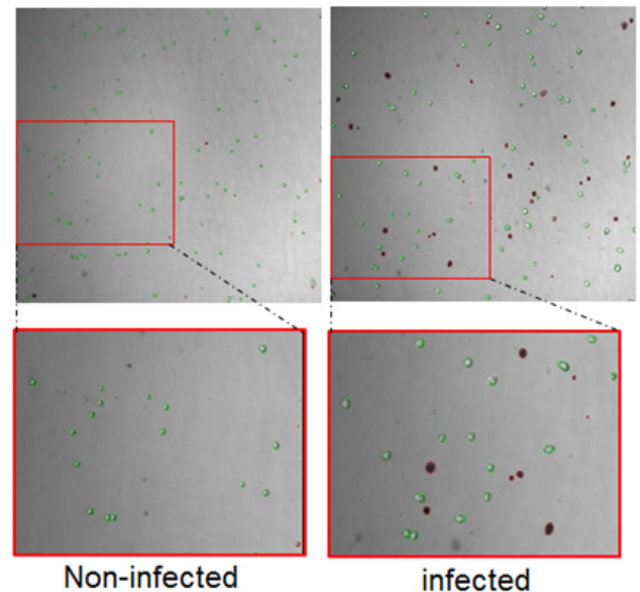

Figure 2: Single images of non-infected vs. 72 hrs post infection Sf21 cells. Counted cells are indicated by green for viable cells and red for non-viable cells

In Fig. 3 the micrograph on the left shows non-infected cells with an average diameter of 14 µm (Figure 3A). The population of viable-cells is displayed in the cell diameter histogram by green bar lines. Upon infection with the recombinant baculovirus the size (mean diameter) of the Sf21 cells increases. This read-out has been used to define the optimal expression time. 48 hours post infection, the average cell size is 18 µm diameter (Fig. 3B), further expression results in an average cell size of 20 µm (Fig. 3C).

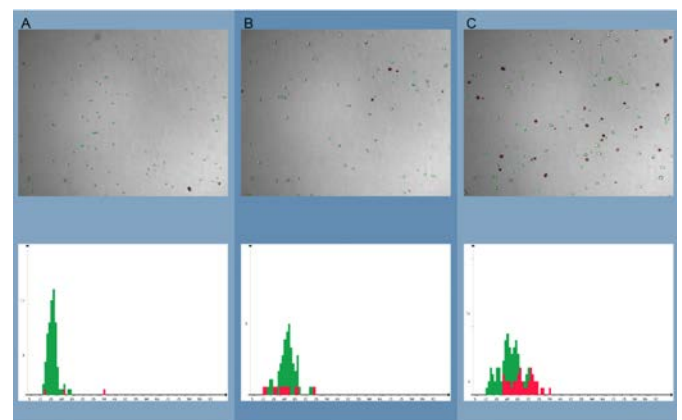

Figure 3: Cell counting and cell diameter histograms from non-infected (A) Sf21 cells, 48 h post transfection (B) and 72 h post transfection (C). The 72 h post-infection cell population has a viability of 71 % compared to the non-infected cells with a viability of 95 %.

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Summary

The Spark 10M cell counting module is fully validated for cell-counting, viability and size distribution assays and can be used with a variety of mammalian and insect cell lines. Users benefit from the easy handling using the one-click solution with pre-set parameters, or high flexibility after setting their own parameters.

References

(1) Jarvis DL; Baculovirus-insect cell expression systems. Methods Enzymol 463:191-222

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