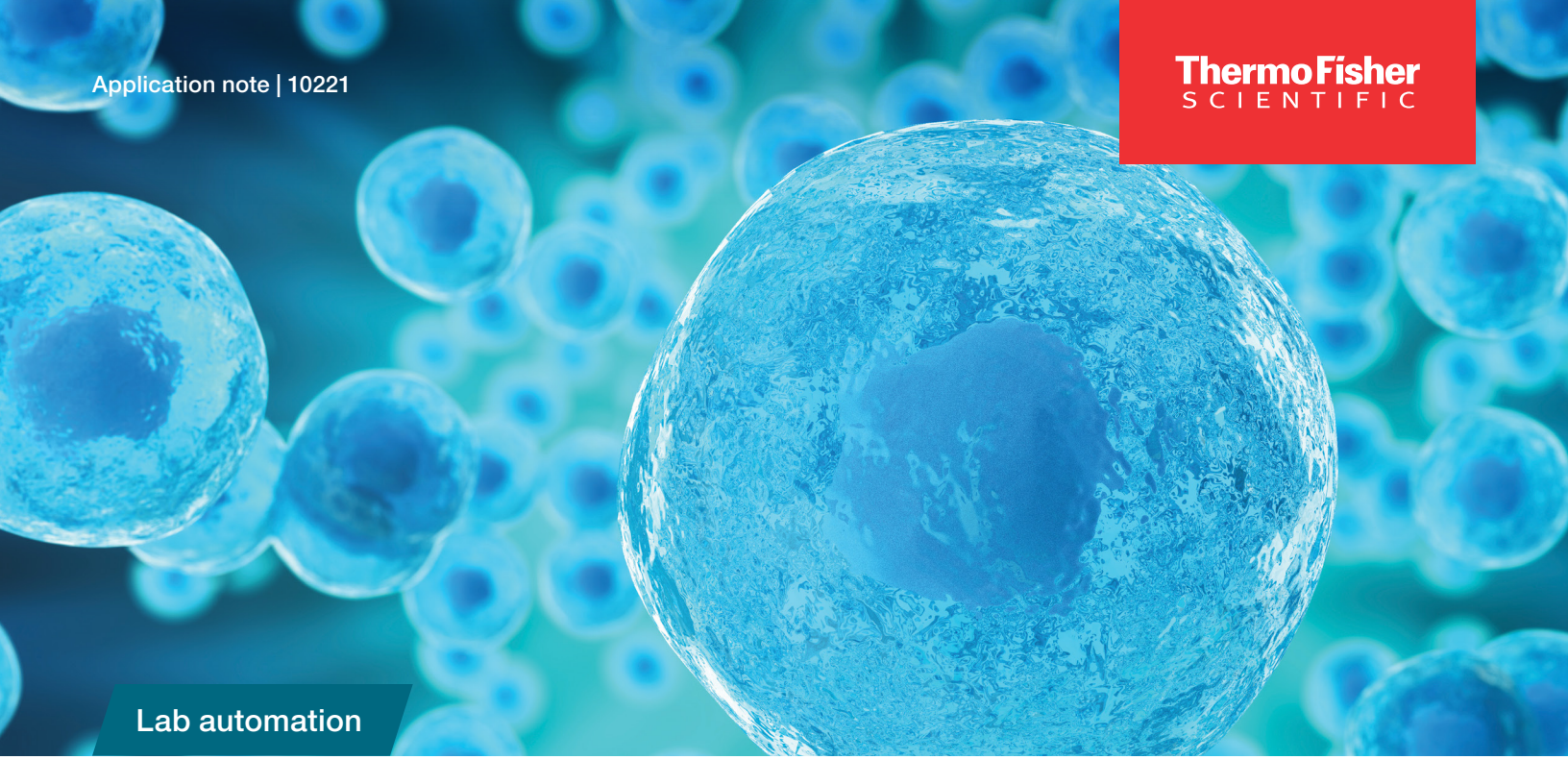
Application note | 10221



Lab automation

Protein Expression Equivalency between automated and offline shaking incubation

Large-volume shaking and incubation performance in automated cell selection and transient transfection of the biotherapeutics workflow

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Keywords

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Goal

To verify that the Thermo Scientific™ Cytomat™ 2 Selector automated shaking incubator is the ideal solution for automating cell growth, transient protein expression, and stable clone selection. To achieve this goal, the development of an automated large-amplitude shaking incubator was needed. This novel, automated incubator simplifies transient protein expression and stable clone selection, streamlining biologics development.

We show that the Cytomat 2 Selector automated incubator has the same performance as offline shaking incubators in the market which are used for transient transfection and stable clone selection in biotherapeutics. We verify that the performance on cell viability, cell density, growth rate, and protein expression is comparable in SLAS (formerly SBS) labware to that of flasks.

Introduction

The stable clone selection process refers to the method by which a particular clone is chosen from a collection of genetically identical cells or organisms. The process can vary depending on the purpose of the cloning and the specific techniques being used. As the first step, a biological target is selected to provoke an immune response and extract the antibody's DNA sequence. The next step of the workflow is to utilize CHO or HEK293 cells to express the antibody of choice.

Transfection is the process of introducing nucleic acids (DNA or RNA) into cells. The introduced nucleic acid sequence may exist in the cell transiently or be integrated stably into the host cell's genome. Cells transiently transfected only express the protein of interest for a limited time, allowing for efficient screening of a large number of proteins. Previously, this workflow had to be performed manually or in smaller scale, SLAS-formatted 96-deep well plates or 24-deep well plates on a 3 mm shaking diameter automated incubator. These steps can now be on full scale automated to improve efficiency, reduce variability, and increase throughput.

The following step for cell line selection needs large amplitude shaking (25 mm shaking diameter) and incubation for several days with a capacity of up to 25 mL per cell line. Previously, there were no automated shaking incubators with this large shaking diameter available. Thus, a manual process with an offline automated shaking incubator and specific SLAS (SBS) labware or flasks was required. The only available automated shaking incubation required splitting cell lines into multiple wells/plates with a small shaking diameter.

The Cytomat 2 Selector automated incubator automated incubator closes this gap with its automated large-amplitude shaking.

It offers a 25 mm shaking diameter and incubation conditions ensuring superior cell growth for cells in suspension. Standard SBS labware (deep well plates or 5–35 mL culture vessels) can be used in the Cytomat 2 Selector automated incubator for this stable clone selection or transient transfection workflow.

The Cytomat 2 Selector automated incubator gives a seamless transition from a manual to an automated shaking incubation process, gaining efficiency and throughput for the clone selection or transient transfection scale-up.

The automated decontamination routine ContraCon is standard to ensure a safe and harmless cleaning procedure in between runs where no hardware needs to be removed. This significantly reduces the risk of contamination.

The automated large amplitude shaking of the Cytomat 2 Selector automated incubator offers the same orbital shaking pattern as the well-known Cytomat 2 Tower shaker (TRUE orbital shaking). Leading offline shaking incubators have a shaking diameter of 25 mm.

The Cytomat 2 Selector automated incubator offers the same shaking diameter of 25 mm and delivers highly reproducible results. Two synchronized shaking drive systems (located at the bottom and top of each stack) provide homogeneous shaking of all plates with consistent shaking amplitude independent of load and position.

The two shaking stackers are individually controlled to run concurrent applications for the highest flexibility. Plates can be unloaded from one stacker while the second remains shaking enhancing process stability. An active plate clamping mechanism holds the plates securely for replicable application results.

Results

A large UK BioPharma company wanted to determine if a Cytomat 2 Selector automated shaking incubator offered comparable CHO growth and transient protein expression conditions in SLAS-formatted 24-deep well and 6-deep well plates to a non-automated shaking incubator and a control flask in a non-automated shaking incubator.

Figures 1 and 2 show the used labware inside the Cytomat 2 Selector automated incubator.

Figure 1. Polystyrene transparent 6-deep well microplate (Enzyscreen.com)

Figure 2. Polypropylene 24-deep well microplate (Porvairsciences.com)

In separate experiments, CHO cell line growth, viability, and protein expression yields were monitored across the different culture formats. A proprietary CHO transient cell line was seeded at a cell density of $3.0e^5$ cells/mL × 30 mL of media in (2) 6-deep well plates (SLAS format) with gas permeable plate seal (HJ-Bioanalytik) and (1) 125 mL control flask.

One 6-deep well plate was placed in a non-automated shaking incubator (i) (25 mm shaking diameter) at 250 rpm, 37°C and 5% CO_2. The second 6-deep well plate was placed in the Cytomat 2 Selector incubator at 210 rpm, 37°C and 5% CO_2.

The control flask was placed into a non-automated shaking incubator (k) at 190 rpm, 37°C and 5% CO_2. Cultures were monitored for cell density and viability to Day 4 with all cultures maintaining 99% viability and reaching 12.0e^6 cells/mL (Figure 3).

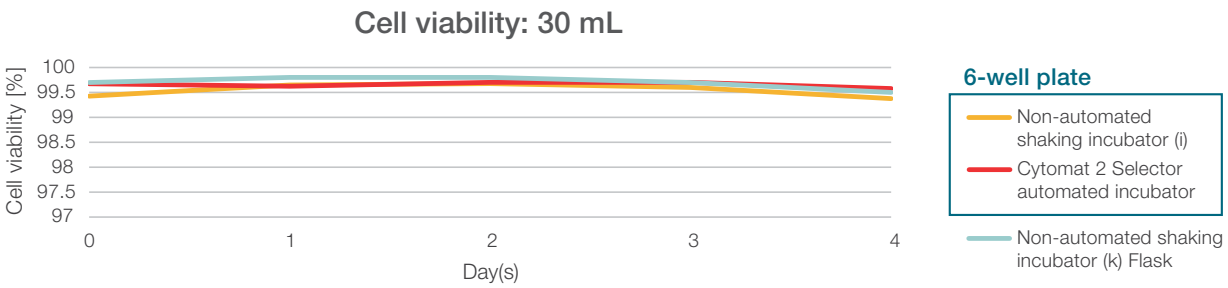

Figure 3. Cell viability with 30 mL inside various labware

Additionally, transient CHO protein expression was evaluated in the 6-well format in the Cytomat 2 Selector automated incubator and non-automated shaking incubator (i). A proprietary CHO transient cell line was transfected in 30 mL of media in (2) 6-deep well plates (SLAS format) with gas permeable plate seal (HJ-Bioanalytik) and (1) 125 mL control flask.

One 6-deep well plate was placed in a non-automated shaking incubator (i) (25 mm shaking diameter) at 250 rpm, 37°C, and 5% CO_2. The second 6-deep well plate was placed in the Cytomat 2 Selector incubator at 210 rpm, 37°C, and 5% CO_2. The control flask was placed into a non-automated shaking incubator (k) at 190 rpm, 37°C, and 5% CO_2. On Day 2 post-seeding, a proprietary CHO feed was administered to all cultures, and a cell density of ~5.0e6 cells/mL and >99% viability was recorded for each culture format (Figure 4).

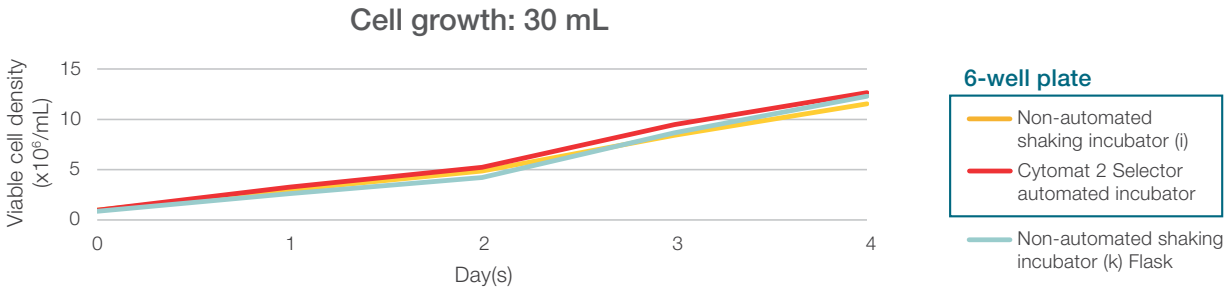

Figure 4. Cell density (cell growth) with 30 mL

All cultures were harvested on Day 7, and the protein was determined by a Protein G HPLC quantitation assay. 6-deep well plates reached ~1,400 mg/L, exceeding the customer's targeted yield while also comparable to the control shake flask data of ~1,700 mg/L (Figure 5).

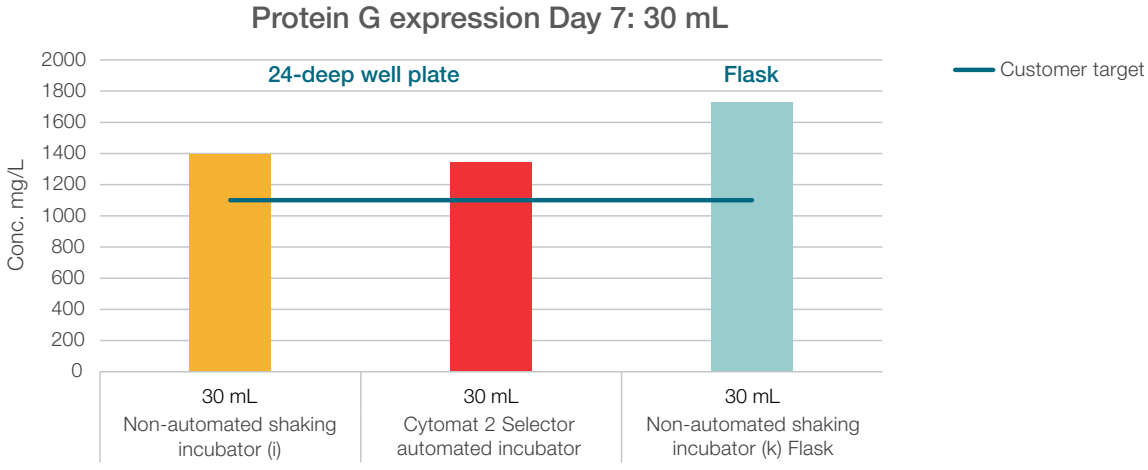

Figure 5. Protein G expression yields with 30 mL

The large UK BioPharma company also evaluated 5 mL transient CHO cell growth, viability (data not shown), and protein expression yields (Figure 6) in SLAS-formatted 24-deep well pyramid-bottom plates with gas permeable seal (HJ-Bioanalytik), achieving comparable results to the 6-deep well plate data above and in comparison to the non-automated shaking incubator.

One 24-deep well plate was placed in a non-automated shaking incubator (i) (25 mm shaking diameter) at 350 rpm, 37°C, and 5% CO_2. The second 24-deep well plate was placed in the Cytomat 2 Selector incubator at 350 rpm, 37°C, and 5% CO_2. The control flask was placed into a non-automated shaking incubator (k) at 190 rpm, 37°C, and 5% CO_2. CHO cell growth was monitored for four days post-seeding at a viable cell density of 3.0e5 cells/mL × 30 mL. A proprietary CHO feed was administered on Day 2 across the three culture conditions. Day 4 viable cell density reached ~12.0e6 cells/mL for the three tested conditions.

CHO cell viability was also measured for the three cultures across a 4-day time course. Each CHO cell culture format maintained >99% viability for the 4-day time course.

On Day 7, the cultures were harvested, and protein titer was determined by a Protein G HPLC quantitation assay. Protein yields for the Cytomat 2 Selector automated incubator and non-automated shaking incubator (i) 24-deep well plates expressing ~1,400 mg/L, exceeding the customer's targeted yield and comparable to the control shake flask data (Figure 6).

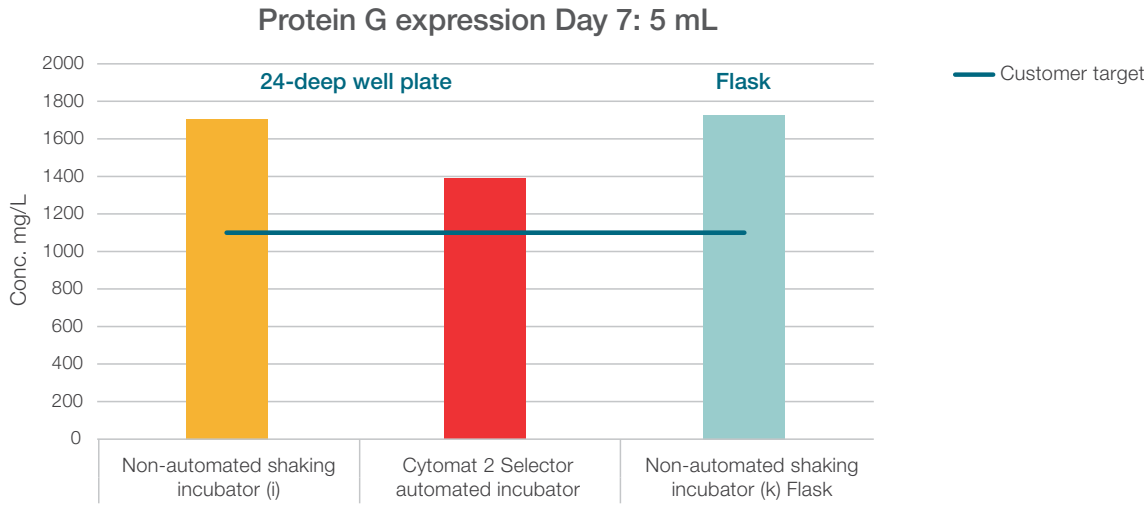

Figure 6. Protein G expression yields with 5 mL

Conclusion

This customer stated the Thermo Scientific Cytomat 2 Selector automated large-amplitude shaking incubator shows comparable results in cell growth and expression to a reference (traditional) manual non-automated shaking incubator in the biotherapeutics workflow.

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