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Scientific Poster

Dramatic Improvement in Recombinant Protein Yields using a 96-well High Throughput High-Speed Shaking Format and the INFORS HT Multitron

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Abstract

A growing demand for recombinant proteins has driven the Biotechnology Industry to develop high throughput production and screening methods. Mammalian cell lines are preferable but have been difficult to adapt to 96-well culture due to their high oxygen and CO_2 demands. By using the Multitron incubator shaker (INFORS HT), with 3 mm orbit at 800–1000 min^{-1}, we have been able to increase recombinant protein yields significantly and improve well-to-well consistency compared to cultures using a 25 mm orbit. These gains can be attributed to improved cell growth and viability because of optimal circulation. Additionally, we investigated different sealing systems and a peg tray that will enable us to stack up to 40 96-deepwell plates per incubator. This will allow for the development of an automatable high-throughput protein expression system that will shave months off of our current development time.

Methods

Cell Culture and Transfection
The 293E cell line, a suspension adapted HEK293 line licensed from the Biotechnology Research Institute, Canada was used in all experiments. Cells were cultivated as a seed stock in spinner flasks under the following conditions: 37°C, 5% CO_2, and 150 min^{-1} agitation speeds. A 1:1 mixture of F-17 (Life Technologies) and SFM4 Transfix (GE Healthcare Life Sciences) was used as both the seed and production medium. Transfections were performed using a 25-kDa linear PEI (Polysciences) and purified plasmid DNA (Qiagen).

Experimental Design
All experiments were conducted on 96-deep well deep well plates (Greiner Bio-One Masterblock), and tested two agitation speeds (800 and 1000 min^{-1}) and two working volumes (500 and 1000 ul). Transfections were performed in bulk at a 50 mL working volume and aliquoted into the final working volume in 96 well deep well blocks. At the indicated time of harvest cultures were removed from the incubators, clarified by centrifugation, and the supernatant samples were analyzed by HPLC for protein expression.

Cell Count and Protein Concentration Measurement
Viable cell density and cell viability were measured using a TC 20 Automated Cell Counter (Bio-Rad Laboratories). Cell culture samples from 96-deep well plates were collected on culture days 0–7 for analysis. For protein concentration determination, supernatant samples assayed in replicate using affinity chromatography (Agilent Technologies).

Evaporation Rate Determination
The evaporation rate with three sealing systems were compared: Duetz-System (Kuehner Technology), gas permeable membrane sealers (Axygen Scientific), and a Microplate box (INFORS HT). Evaporation was determined by measuring the difference in mass of each plate over 5 days calculated via linear regression and divided by 96 to generate a mg/well*day value. Plates were filled with 1000 ul per well and agitated at 1000 min^{-1} at a 3 mm orbital diameter in an 75% humidity incubator.

Conclusion

By using the INFORS HT Multitron incubator shaker with a 3 mm orbital diameter, we have been able to increase recombinant protein yields significantly and improve well-to-well consistency compared to cultures using a 25 mm orbit. These gains can be attributed to improved cell growth and viability because of optimal circulation. Additionally, we tested two agitation speeds, two working volumes, and three plate sealing formats. The optimal parameters were identified to be a fill volume of 1000 ul and an agitation rate of 1000 min^{-1} using a 3 mm orbital diameter. By using a membrane-based sealer with the peg tray we were able to maximize throughput in our process. Although other sealing options could have the added benefit of reduced evaporation. Together these findings allow for a high throughput platform for recombinant protein expression.

Reference

[1] www.thermoshaker.com/?tag=orbital-shaker
[2] Bos, A.B., Luan, P., Duque, J.N., Reilly, D., Harms, P.D., Wong, A.W. (2015) Biotechnology and Engineering. 112 (9) 1832-1842.

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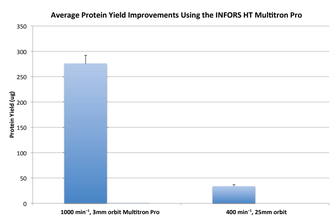
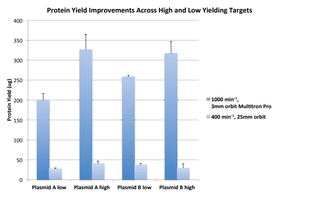
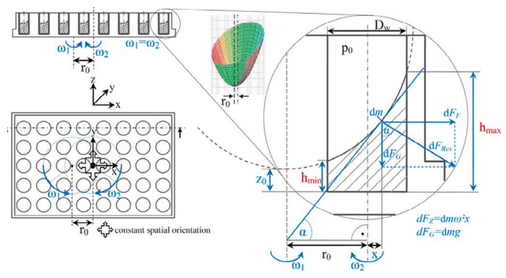
Figure 1:
Recombinant protein yield improvements using the INFORS HT Multitron incubator shaker with 3 mm orbital compared to a 25 mm orbital shaker.



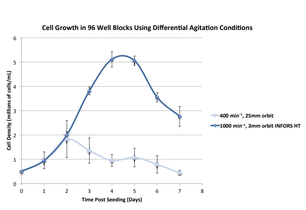
a) Initial experiments utilizing a 25 mm shaker inside of an incubator resulted in average recombinant protein yields of 33 ug/ml. Using the INFORS HT Multitron incubator shaker, average yields increased dramatically to 276ug/ml.



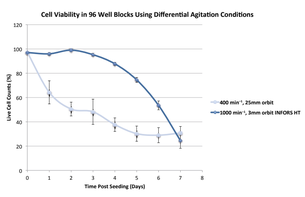


b) Further analysis of different high and low yielding targets confirmed that the effect was consistent for multiple proteins.

Figure 2:
Increased oxygen transfer enhances cell growth

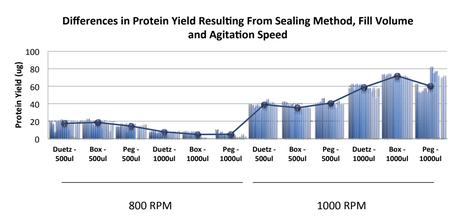


a) Cells exhibited typical growth patterns in the INFORS HT Multitron incubator shaker comparable to large-scale culture in spinner flasks. Cells grown using a 25 mm orbit shaker at 400 min^{-1} failed to grow appreciably. This effect was most likely due to differences in oxygen transfer[2].



b) Cell viability using the INFORS HT Multitron incubator shaker was comparable to that seen in large-scale spinner flasks. In contrast, cell viability using a 25 mm orbit shaker at 400 min^{-1} dropped appreciably one day post seeding and continued to decrease for the duration of the experiment.

Figure 3:
Differences in protein yield resulting from sealing method, fill volume and agitation speed

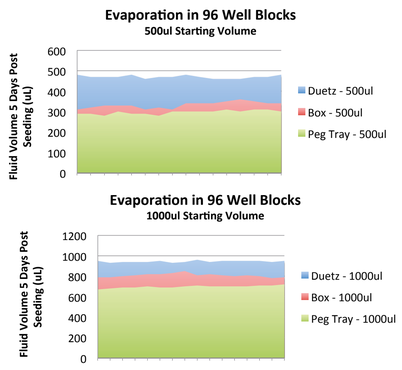


a) No significant differences in protein yields were observed using the three sealing methods.

b) Over all conditions, the protein concentrations were higher in wells filled with 500 ul of total culture compared to those filled with 1000 ul (Data not shown). However, when taking into account the relative differences in fluid volume, the total yields observed in the 1000ul culture were significantly higher.

c) Across all conditions, increasing agitation speed from 800 min^{-1} to 1000 min^{-1} increased protein yields.

Figure 4:
Evaporative loss using different sealing formats



A comparison of three commercially available plate-sealing formats resulted in significant differences in the residual fluid volume following five days of culture in the INFORS HT Multitron. The least evaporative loss was seen using the Duetz-System followed by the Microplate Box (INFORS HT). Membrane based sealers resulted in the highest level of evaporative loss which was roughly 30–40% depending on the various culture formats.

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