Scalable Transient CHO Method that in 7-12 days may become Stable Cell Pools & Stable Lines for Quantities needed in Toxicology Studies

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ABSTRACT

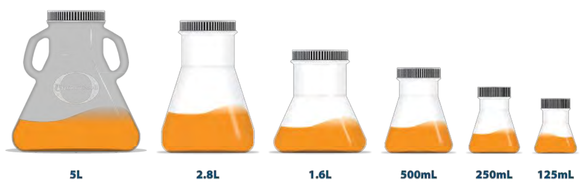
- Increasing competition in recombinant antibody and protein drug discovery has resulted from the U.S. Patent Office instituting 'First to File'.
- As a result, the time frame for generating patentable biologic molecules has been extremely compressed. Companies are now forced to utilize methods that can significantly speed transient hits through to stable pools and clones.
- Thomson Instrument Company and MaxCyte will show new methodology to produce scalable recombinant antibodies and proteins that will lead to faster and higher titers with excellent glycosylation.
- Our technical advancements in shake flask design, and improvements in transfection significantly shorten timelines and improve product quality allowing for faster filing.

INTRODUCTION

- Recombinant cell lines are a key resource for toxicology studies. Transient transfection quickly expresses the protein of interest, but for difficult to express proteins or in cases where protein homogeneity is essential, it may be necessary to generate stable pools or clonal cell lines.
- The development of stable cell pools (SCPs) decreases heterogeneity but SCPs can take 2 - 3 months to develop using traditional methods.
- Thomson Instrument Company's Optimum Growth™ Flasks and Transfer Caps used in conjunction with MaxCyte's Flow Electroporation transfection technology cut the time taken to develop SCPs down to 7 - 12 days.
- Both the Optimum Growth™ Flasks and MaxCyte Transfection System are compatible with a variety of cell types which provides flexibility, and ensures that the data obtained are relevant to the specific application Table 1 and Figure 1).

MATERIALS & METHODS

- Both the Optimum Growth™ Flasks and MaxCyte Transfection System are compatible with a variety of cell types which provides flexibility, and ensures that data obtained are relevant to the specific application (Figure 1).

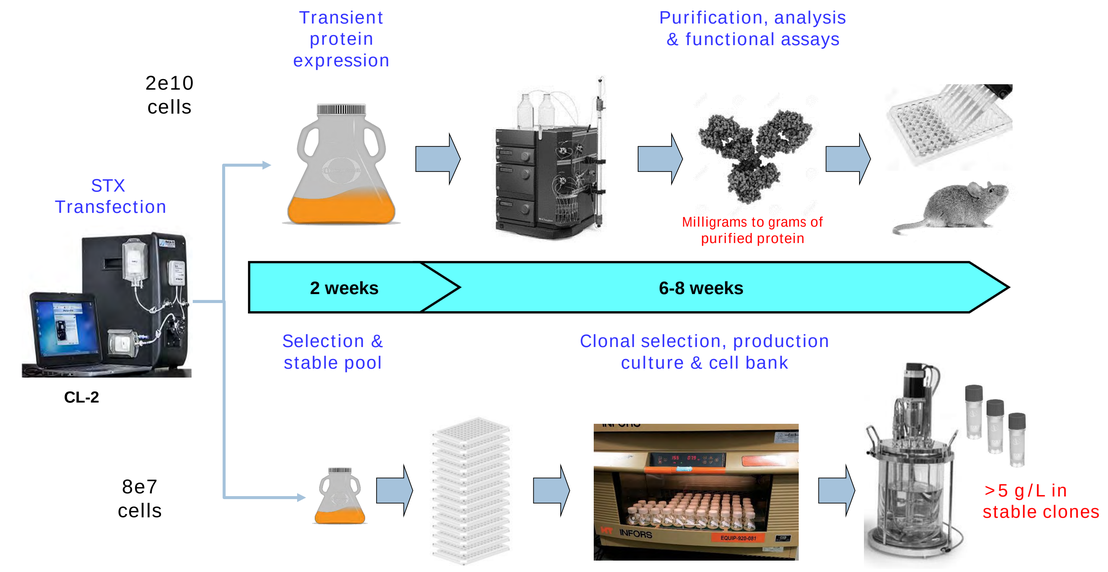


Stop by BOOTH 511 for Shake Speeds & Fill Volumes

fig.1 Process for growing CHO cells in 7-12 days.

1. Suspend cells in MaxCyte buffer (1e8-2e8 cells/mL) + DNA/RNA/Protein (1-2 µg/1e6 cells)
2. Transfer cells to sterile, single-use processing assembly (PA)
3. Attach PA to instrument, select protocol & click "start" button
4. After 30-40 min. recovery, transfer cells to Optimum ™ Flask culture with 1% PF-68

fig.2 Transfection with the MaxCyte Scalable Transfection System produces milligram to gram quantities of protein within 2 weeks via large-scale transient expression. In parallel, stable pools can be generated in the same amount of time by applying selection to a subset of the transient culture.



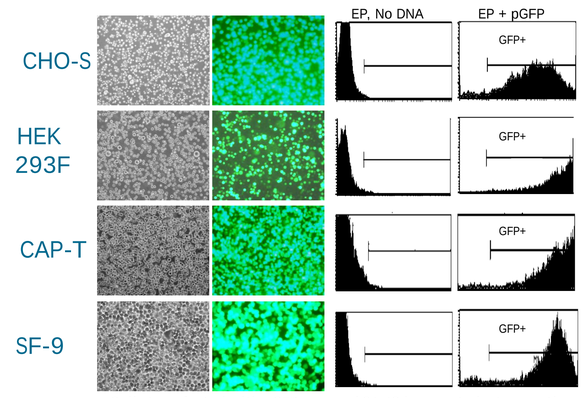
RESULTS

- Use of Optimum Growth™ Flasks and Transfer Caps for cell growth before and after transfection using the MaxCyte System results in high levels of cell viability and transfection efficiency, which lead to increased protein expression and consistent protein quality and uniform glycosylation over many generations.
- Thomson flasks come in a range of sizes and formats that are a perfect match for MaxCyte's transfection scalability. The MaxCyte STX® Scalable Transfection System can transfect 0.5e6 to 2e10 cells at one time. Transfected cells can be transferred directly to Thomson flasks, which accommodate volumes from 20mLs to 3.2L .

MaxCyte provides high transfection efficiency and high viability, which are essential for robust protein expression

- MaxCyte provides >95% transfection efficiency and viability with cells commonly used for bio-production, including CHO cells and insect cells
- No restrictions on cell lines, expression vectors, media or culture vessels

fig. 3 Transfection efficiency of pGFP post electroporation.



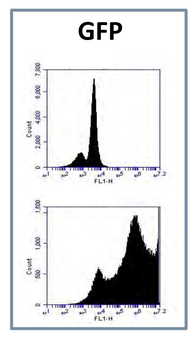
Cells transfected with pGFP assayed 24-48 hrs. post electroporation

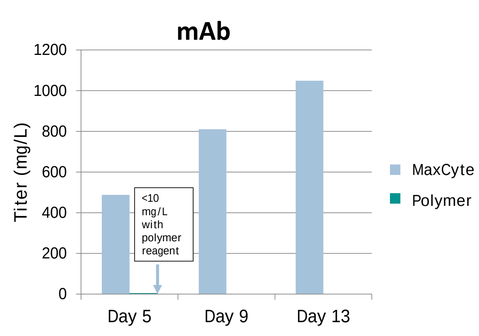
>1 Gram/Liter Transient Antibody Expression in CHOK1SV Cells

- CHOK1SV cells transfected with GFP and IgG expression plasmids showed high transfection efficiency and high titer antibody expression, respectively.
- Parallel transfections with a polymer-based transfection reagent yielded nearly undetectable antibody titers.
- MaxCyte's technology is compatible with multiple CHO cell strains.

fig. 4 CHOK1SV showed high transfection efficiency and titers

12 Optimum Growth™ 2.8L Flasks with 1.4L

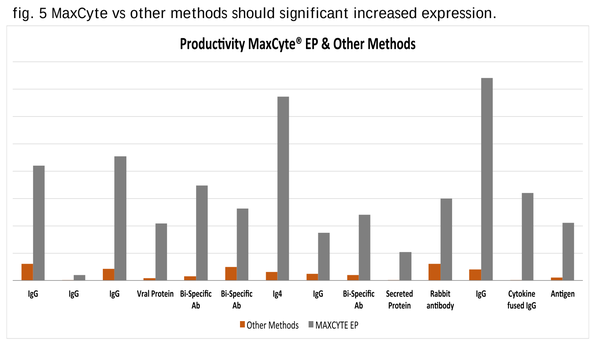




MaxCyte vs Other Transfection Methods: Dramatically Increased Transient Expression with Multiple Proteins and Cell Types

18 Optimum Growth™ 1.6L Flasks with 900mL

fig. 5 MaxCyte vs other methods should significant increased expression.



Customer-generated data

CONCLUSIONS

- Used together, Thomson Instrument Company's Optimum Growth™ Flasks and MaxCyte's Flow Electroporation Transfection technology rapidly generate milligram to gram quantities of Protein via Transient Expression while simultaneously producing stable pools and clones.
- MaxCyte flow electroporation allows large-scale, high titer, transient protein in CHO cells for rapid generation of activity data and physical characterization.
- In parallel, a subset of cells from the transient culture can be subjected to selection for stable pool or clone generation.
- High transfection efficiency and post transfection viability enable production of stable pools within 14 days and identification of high titers clones within 8 weeks of transfection.
- Gram quantities of uniform glycosylated protein for SCP and clone generation.
- Using this system SCPs can be generated in as little as 7 - 12 days.