Strategies for High-Titer Protein Expression Using the ExpiCHO and Expi293 Transient Expression Systems

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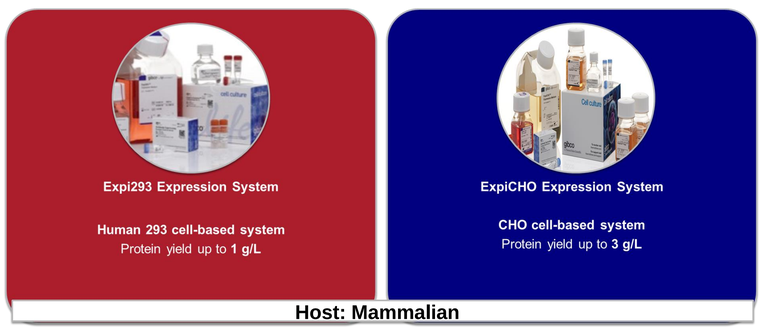
Abstract and introduction

The Expi293 and ExpiCHO transient expression systems offer all-in-one solutions for generating high-titer recombinant proteins for a broad range of research applications including candidate drug identification, reagent production, structural biology and vaccine research and membrane protein biology. While both Expi systems offer the ability to generate high levels of recombinant proteins, the inherent differences between HEK293 cells and CHO cells make these systems differentially applicable for various protein expression needs. Here, we present the latest data on the Expi293 and ExpiCHO expression systems as well as suggested paradigms for instances where either Expi293 or ExpiCHO would be most applicable for a given research requirement.

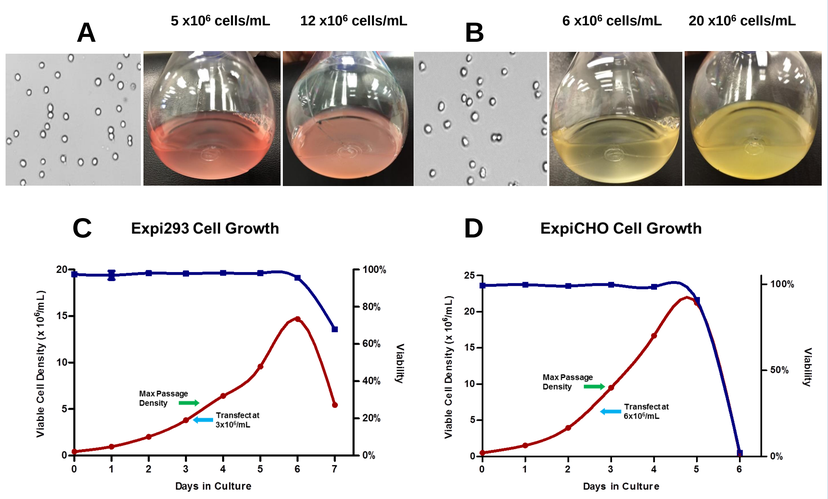


What makes the Expi systems unique?

Complete, optimized systems comprising:

- Cells
- Expression media
- Transfection reagents
- Expression enhancers
- Feeds
- DNA vectors

Expi293F and ExpiCHO-S Cell Lines



Expi293F cell line attributes

- Derived from FreeStyle 293F cells
- Adapted for high-density culture (≥15M cells/mL)
- Doubling time ~24-25 hours
- Cell diameter 18 - 20µm (culture – expression)
- Highest transfection efficiency (80-85+%)
- Stable growth and expression profiles over 30 passages
- High quality, biologically-active protein

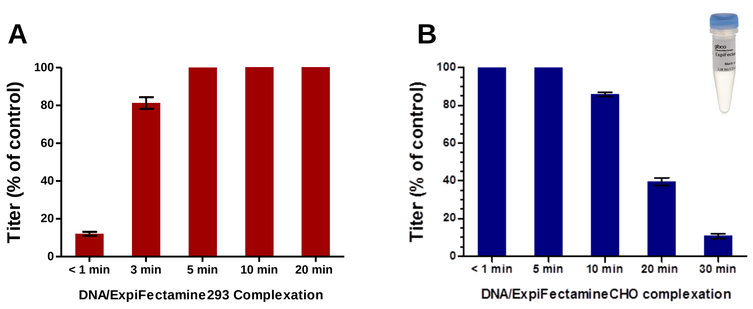
ExpiCHO-S cell line attributes

- Derived from GMP CHO-S cells
- Adapted for high-density culture (≥20M cells/mL)
- Doubling time ~17-18 hours
- Cell diameter 14 - 20µm (culture – expression)
- High transfection efficiency (75-80%)
- "CHO-like" glycosylation profiles to match stable bioproduction
- High quality, biologically-active protein

Figure 1. Characterization of Expi293F and ExpiCHO-S cells.

(A) Expi293F cells. **(B)** ExpiCHO-S cells. **(C)** Growth and viability curves for Expi293F cells grown in standard shake flask culture. **(C)** Growth and viability curves for ExpiCHO-S cells grown in standard shake flask culture.

Expi293 and ExpiCHO Transfection kits



Expi293F transfection kit

- Vector pcDNA3.4
- DNA: recommended 1µg/mL
- ExpiFectamine 293: 3.2µl/mL of culture to transfect
- Complexation medium: OptiMEM
- Complexation timing: ~5-20 minutes

ExpiCHO-S transfection kit

- Vector pcDNA3.4
- DNA: recommended 0.8µg/mL
- ExpiFectamine CHO: 3.2µl/mL of culture to transfect
- Complexation medium: OptiPro SFM
- Complexation timing: ~1-5 minutes

Figure 2. Comparison of transfection conditions for Expi293 and ExpiCHO

(A) Expi293F DNA and ExpiFectamine 293 complexation timing. **(B)** Expi293F DNA and ExpiFectamine 293 complexation timing

Expi293 and ExpiCHO Feed and Enhancers

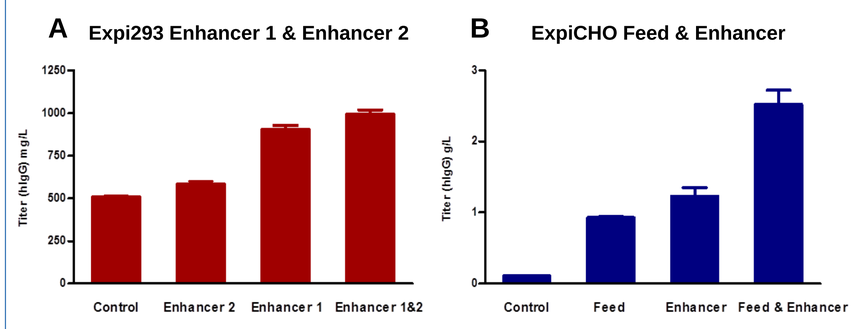


Figure 3. Effects of Expi293 and ExpiCHO feed and enhancers

(A) Protein titers are significantly increased with the addition of the ExpiFectamine293 enhancers**. (B)** Protein titers are significantly increased with the addition of the ExpiFectamine CHO enhancer. and ExpiCHO feed.

Optimal Antibody Expression in Expi293 and ExpiCHO

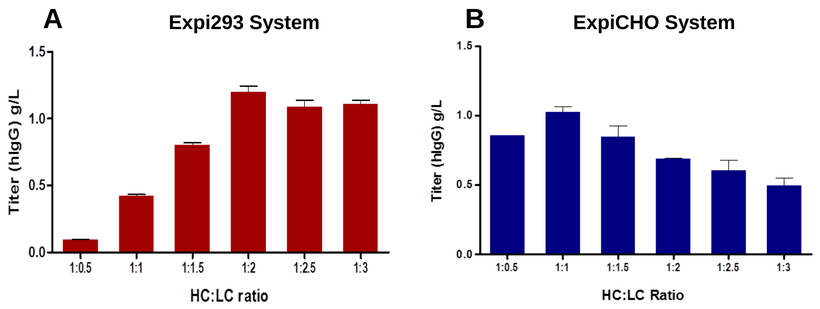


Figure 4. Effects of Expi293 and ExpiCHO feed and enhancers

(A) Optimal antibody expression was observed at 1:2 ratio of heavy and light chain in Expi293 system**.** **(B)** Optimal antibody expression was observed at 1:1 ratio of heavy and light chain in ExpiCHO system.

Plasmid DNA Requirement in Expi293 and ExpiCHO

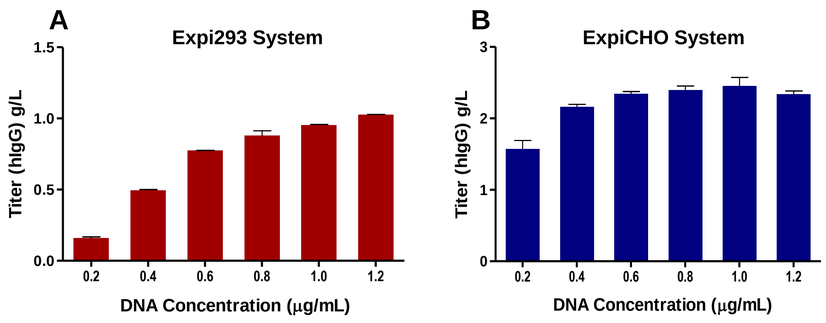


Figure 5. Kinetics of hIgG expression, viability, and viable cell density.

(A) Expi293 system requires the industry standard of 1.0 µg/mL plasmid DNA . **(B)** Despite the high density of cells at the time of transfection, ExpiCHO system requires plasmid DNA as low as 0.6 µg/mL of culture volume generate maximal protein titers.

Expi293 and ExpiCHO hIgG Expression Kinetics

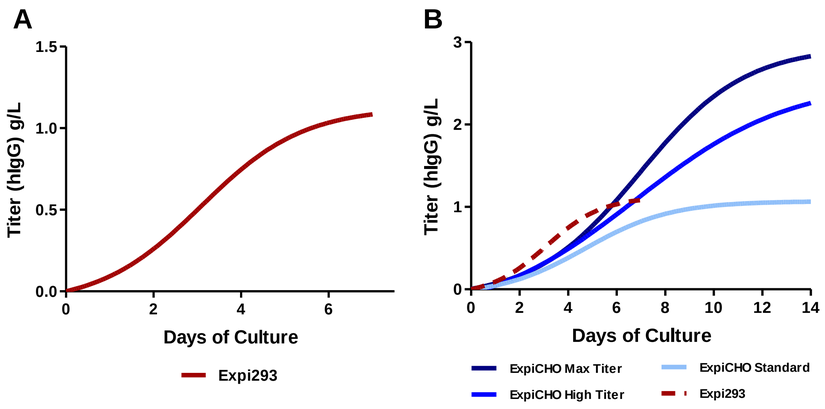


Figure 6. Kinetics of hIgG expression in Expi293 and ExpiCHO systems

(A) Expi293 system requires the industry standard of 1.0 µg/mL plasmid DNA . **(B)** Despite the high density of cells at the time of transfection, ExpiCHO system requires plasmid DNA as low as 0.6 µg/mL of culture volume generate maximal protein titers.

Antibody Purification in Expi293 and ExpiCHO

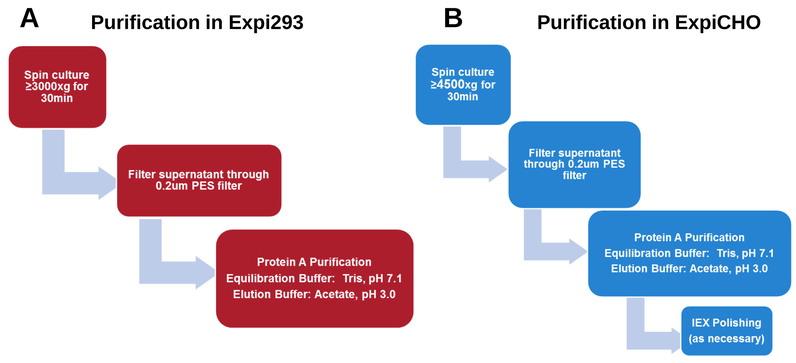
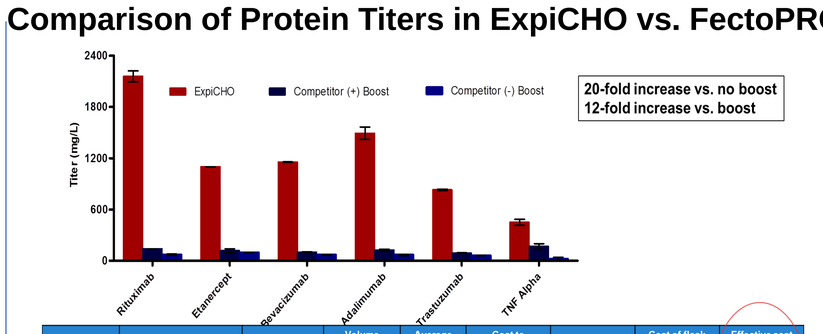


Figure 7. Antibody clarification and purification in Expi293 and ExpiCHO

(A) Antibody clarification and purification workflow in Expi293 system. **(B)** Antibody clarification and purification workflow in ExpiCHO system, IEX polishing step may be required for some high yield proteins.

Comparison of Protein Titers in ExpiCHO vs. FectoPRO®



Cells	Transfection Method	Transfection Reagent/Kit Cost (List $)	Volume transfected per TFXN reagent unit	Average fold titer increase (w/boost)	Cost to transfect 1L equivalent (A)	ExpiCHO Media Costs (B)	Cost of flask and filter for 1L equivalent (C)	Effective cost for equivalent mg protein (A+B+C)
ExpiCHO	Competitor	$375	417mL[1]	20X (12X)	$900 (1000mL)	$336 (1L)	$47[2]	**$1283 ($712)**
ExpiCHO	ExpiFectamine Kit	$750	1000mL		$38 (50mL)	$17 (50mL)	$16[3]	$71

1: Assumes use of 2.4µL Competitor/mL to transfect per MFG CHO protocol
2: Assumes use of 1 x 3L flask and 1 x 1000mL filter
3: Assumes use of 1 x 250mL flask and 1 x 500mL filter

Figure 8. Comparison of Protein Titers in ExpiCHO and FectoPRO®

Protein characterization in Expi293 and ExpiCHO

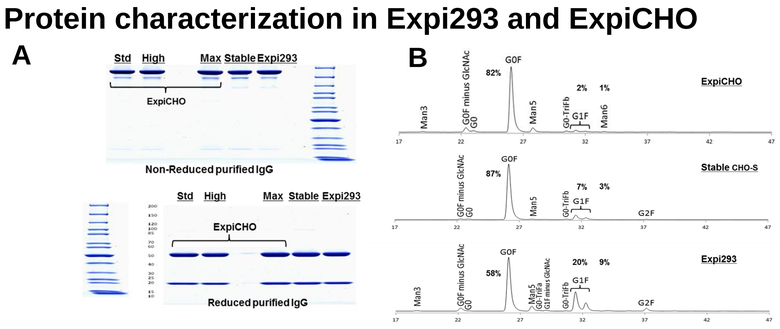


Figure 9. Protein quality and glycosylation patterns in ExpiCHO and Expi293

(A) Human IgG SDS PAGE. **(B)** Human IgG glycans

Expi293 and ExpiCHO Expression Data

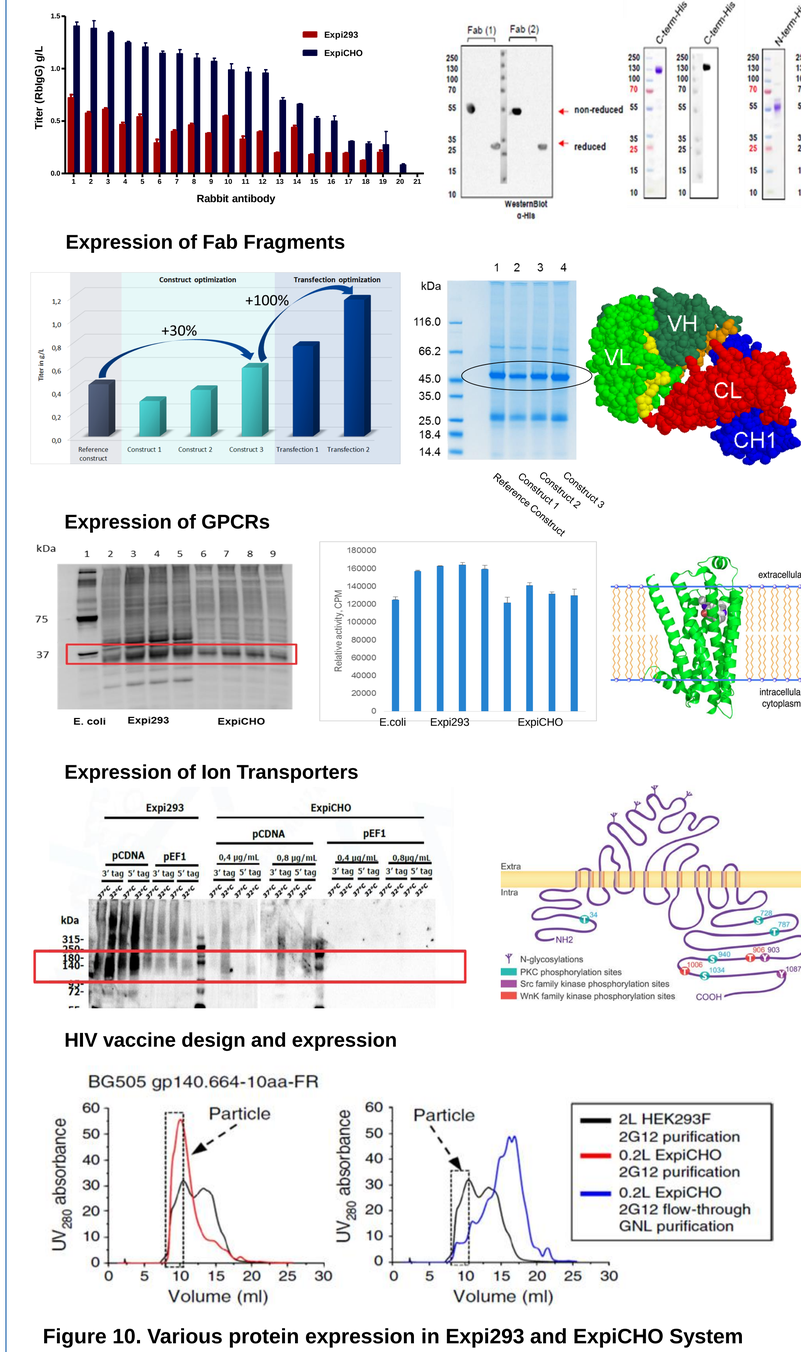


Figure 10. Various protein expression in Expi293 and ExpiCHO System

(A). Antibody expression. **(B)** His-tag protein expression. **(C)** GPCRs expression. **(D)** Fab fragment s expression. **(E)** Ion transporters expression . **(F)** HIV vaccine design and expression.

Conclusions

Expi293 System	ExpiCHO System
Average 4.5-fold improvement in protein yields vs. legacy 293 systems	Up to 100-fold improvements in protein yield vs. legacy CHO systems
Focus on antibody screening, antigen, membrane proteins, vaccines, cell therapy and structural biology	Strong focus on antibodies, Fc fusion proteins, bispecifics
Now available: Expi293 cGMP-banked cells	Streamlines research to bioproduction
Future focus: Expi293 structural biology reagents	Coming soon: cGMP-banked ExpiCHO cells