ExpiSf™ Expression System :A Chemically-Defined Baculovirus-Based Expression System for Enhanced Protein Production in Sf9 Cells

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INTRODUCTION

Here, we present data on the performance of a novel Sf9-based Baculovirus expression system based upon a yeastolate-free, animal origin-free, chemically-defined, high-density culture medium that allows for Sf9 cells to reach densities nearly twice as high as those attained in traditional yeastolate-containing media. Additionally, Sf9 cells adapted to grow to high densities in the yeastolate-free media were generated and a new, high-efficiency bacmid transfection reagent was developed to allow for the generation of high titer baculovirus stocks. Together, with the addition of protein expression enhancer these improvements allow for the optimization of a new expression protocol that takes advantage of the high cell densities achievable with the new chemically-defined medium and adapted Sf9 cells, as well as high multiplicity of infection (MOI), to significantly improve protein titers and enable lot -to- lot consistency of both cell growth and protein expression in a defined media formulation.

Figure 1. System based approach to optimize Baculovirus-based protein expression system

I. Consistency of ExpiSf™ CD Medium

ExpiSf™ CD Medium Attributes:

- Yeastolate free and Chemically-defined (CD)
- Animal origin-free (AOF), serum-free and protein-free
- No supplementation required
- One media for virus generation and protein expression
- Manufactured under cGMP
- Consistent cell growth and protein expression over multiple media lots
- Consistent Performance for over 12 months
- Formulated for high density Sf9 cell growth

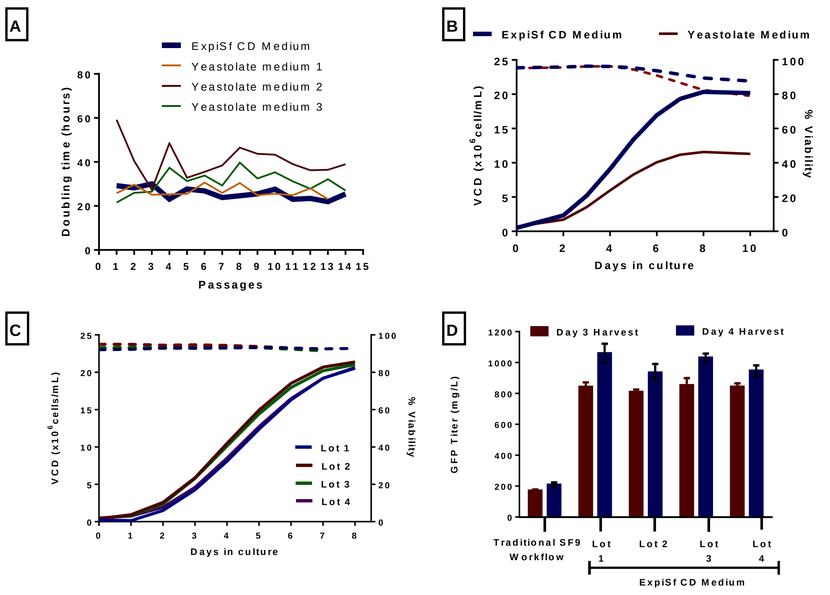

Figure 2. Characteristics of ExpiSf9 CD Medium

(A) ExpiSf CD Medium (blue line) shows more consistent doubling time over 14 passages compared to three other yeastolate containing media. **(B)** ExpiSf CD Medium (Blue line) have higher peak cell densities (~20x10^6 cells/ml) compared to yeastolate Medium (Red line).
(C) Consistent growth in the ExpiSf Media across 4 different media lots.
(D) Consistent protein expression in the ExpiSf Media for over 4 ExpiSf CD Medium lots

II. ExpiSf9™ Cells adapted in ExpiSf™ CD Medium

ExpiSf9™ Cells cell line attributes:

- Adapted for high-density culture in Chemically Defined Medium
- ~24 hour doubling time
- Optimized for high-density infections
- Stable growth and expression profiles over 25+ passages

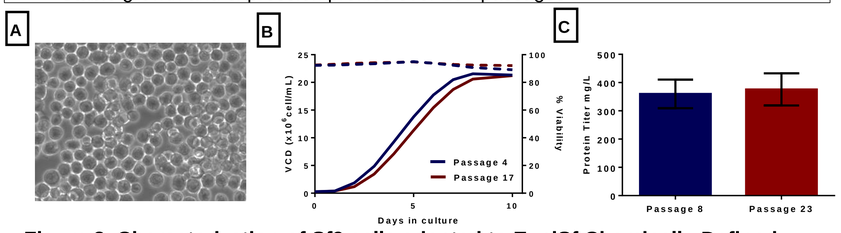

Figure 3. Characterization of Sf9 cells adapted to ExpiSf Chemically-Defined medium

(A) ExpiSf9 cells morphology
(B) Consistent growth over passages. Lines represents the growth of ExpiSf9 cells in ExpiSf CD Medium at passage 4 (Blue line) and passage 17 (Red Line) **(C)** Consistent Protein Expression over passages. Protein titers at passage 8 (Blue Bar) and passage 23 (Red Bar)

III. ExpiFectamine™ Sf Transfection Reagent

ExpiFectamine™ Sf Transfection Reagent

- Convenient protocol
- Scalable virus production in adherent and suspension format

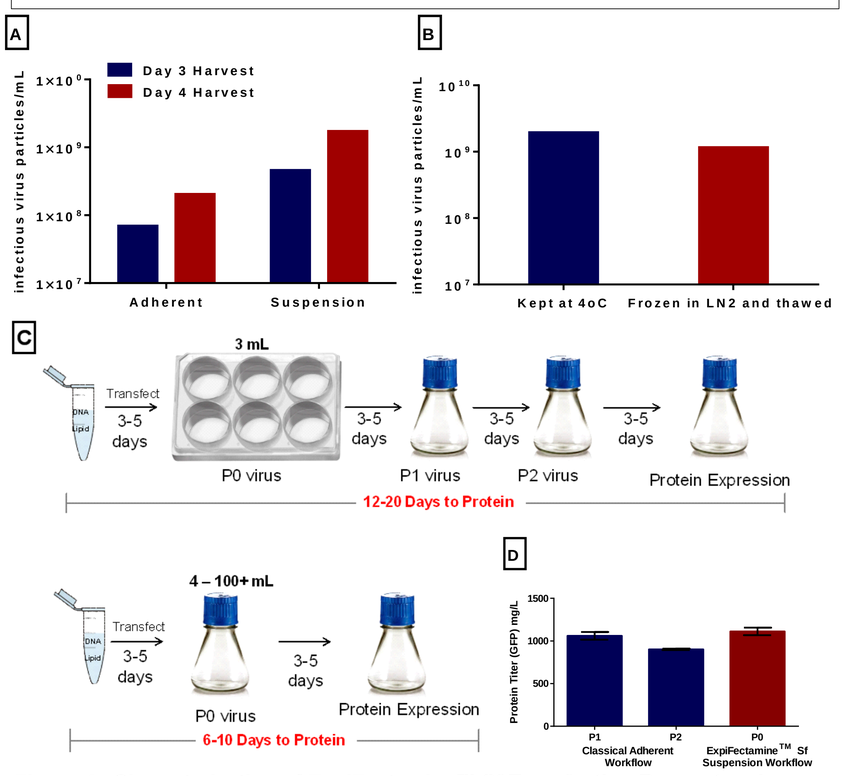

Figure 4. Characterization of ExpiFectamine™ Sf Transfection Reagent and baculovirus generation

(A) Baculovirus Titers obtained at Day 3 and Day 4 from Adherent and Suspension Protocol
(B) Baculoviruses can be frozen at -80 or LN2 for longer storage. Slight reduction in titer is typically observed, but when accounted for it does not affect protein expression
(C) Optimized suspension protocol allows for reduction of the time to protein in half
(D) Equivalent protein titers can be obtain by using P0 from ExpiFectamine Sf compared to P1 or P2 from classical adherent workflow

IV. Characterization of ExpiSf™ Enhancer

ExpiSf™ Enhancer Attributes:

- Essential for obtaining high protein titers
- Needs to be added18-24hr before infection
- Optimized for ExpiSf CD Medium

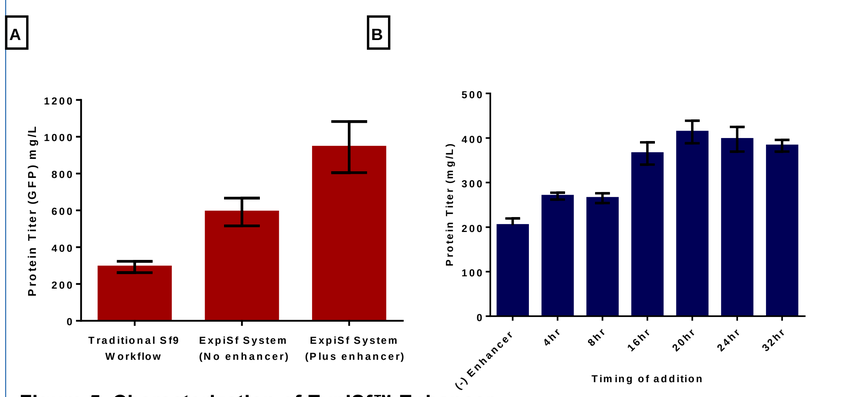

Figure 5. Characterization of ExpiSf™ Enhancer

(A) ExpiSf Enhancer, used in conjunction with ExpiSf CD Medium and ExpiSf9 cells, generated 3-fold higher GFP titers than a traditional Sf9 workflow; **(B)** Addition of ExpiSf Enhancer 18-24hr prior to infection gives the highest protein titer improvement.

V. Protein Expression Workflow

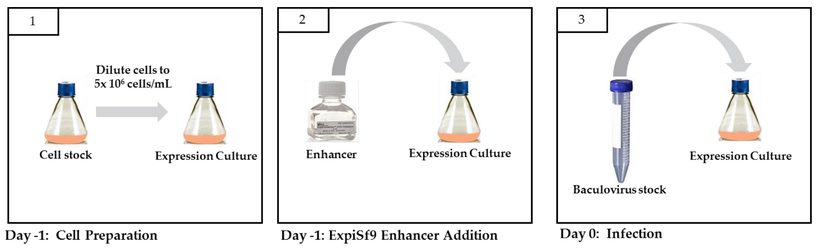

Figure6. Protein Expression Workflow

VI. Kinetics of protein production

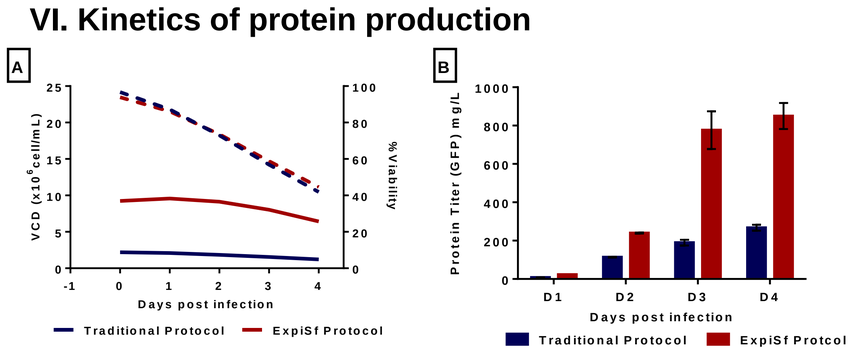

Figure 7. Kinetics of protein production in ExpiSf System

(A) Post-infection cell viability and density decrease comparably in traditional protocol(Blue Line) and ExpiSf System (Red Line) **(B)** Protein production over the course of infection in traditional protocol (Blue Bars) and ExpiSf System (Red Bars)

VII. ExpiSf System Scalability

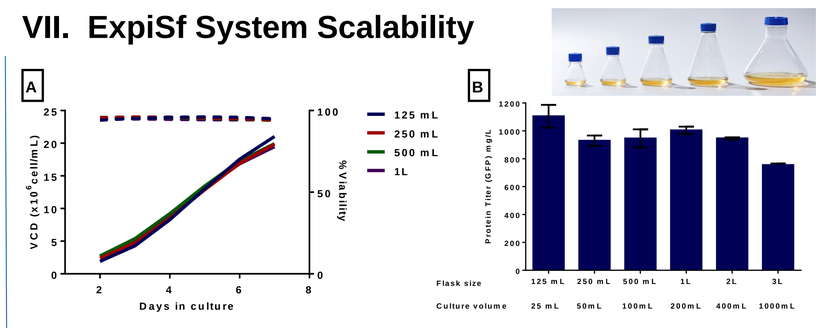

Figure 7. ExpiSf System can be scaled up in variety of shake flask sizes

ExpiSf System is directly scalable from 125mL to 3L flask size. Comparable cell growth **(A)** and protein expression **(B)** were achieved at 125 rpm shake speed.

VIII. ExpiSf System vs Traditional Sf9 Systems

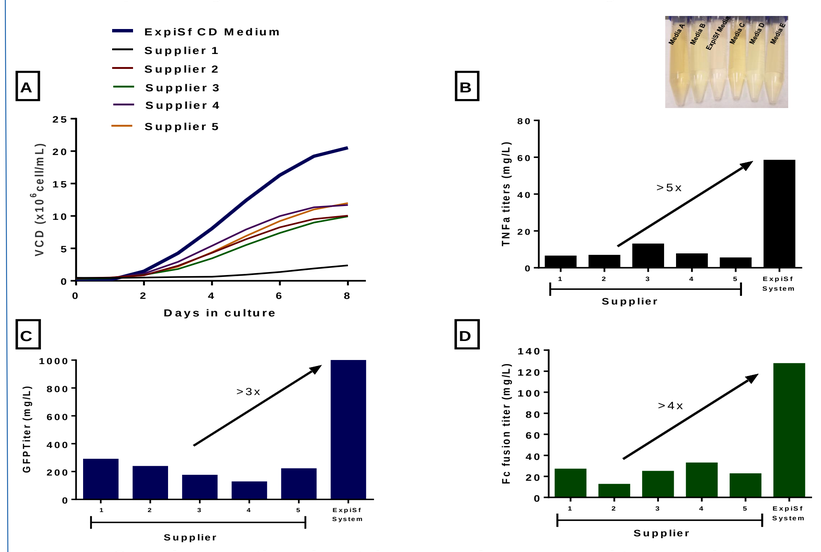

Figure 7. Superior growth and protein expression compared to yeastolate containing medium

(A) Cell growth of ExpiSf9 cells in ExpiSf CD Medium (Blue Line) and four different yeastolate containing medium **(B)** Expression of TNFα in five yeastolate containing medium and ExpiSf System (Last Bar) **(C)** Expression of GFP in five yeastolate containing medium and ExpiSf System (Last Bar) **(D)** Expression of Fc-Fusion protein in five yeastolate containing medium and ExpiSf System (Last Bar)

IX. ExpiSf System vs High Five cells

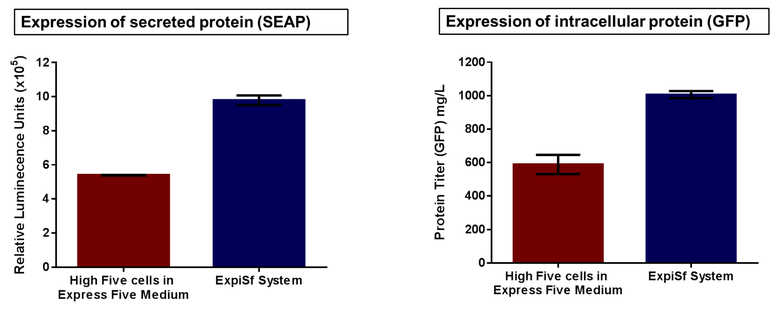

Figure 8. Protein expression in ExpiSf System and High Five cells

(A) SEAP activity in High Five cells in Express Five™ SFM and in the ExpiSf System
(B) GFP protein titers in High Five cells in Express Five™ SFM and in the ExpiSf System

X. Protein Characterization in ExpiSf System

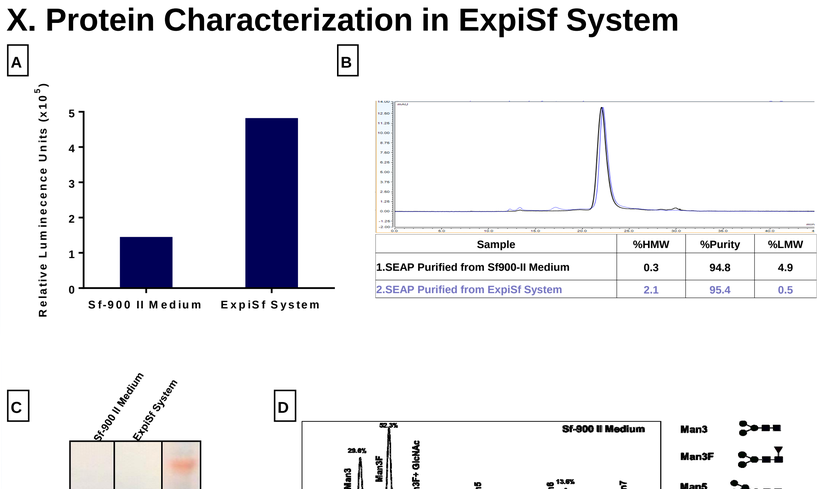

Figure 9. Expression and Purification of Secreted Alkaline Phosphatase (SEAP)

(A) SEAP activity measured by chemiluminesence assay for protein expressed in Sf-900 II Medium and ExpiSf System **(B)** Size Exclusion Chromatograph of SEAP Purified from Sf-900 II Medium and ExpiSf System **(C)** SDS-PAGE of SEAP purified from Sf-900 II Medium and ExpiSf System **(D)** Glycan profiles of SEAP expressed in Sf-900 II Medium and ExpiSf System

CONCLUSIONS

This poster described a system-based approach for enhancing levels of protein production in insect cells using Bac-to-Bac generated viruses that allows for production of proteins at levels exceeding those of the most of the popular systems used at the moment. Using the ExpiSf System, allows for achieving consistent results in a shorter time.

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