

# A Chemically-Defined Baculovirus-Based Expression System for Enhanced Protein Production in Sf9 Cells

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## INTRODUCTION AND ABSTRACT

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 in Sf9 Cell Culture

### 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

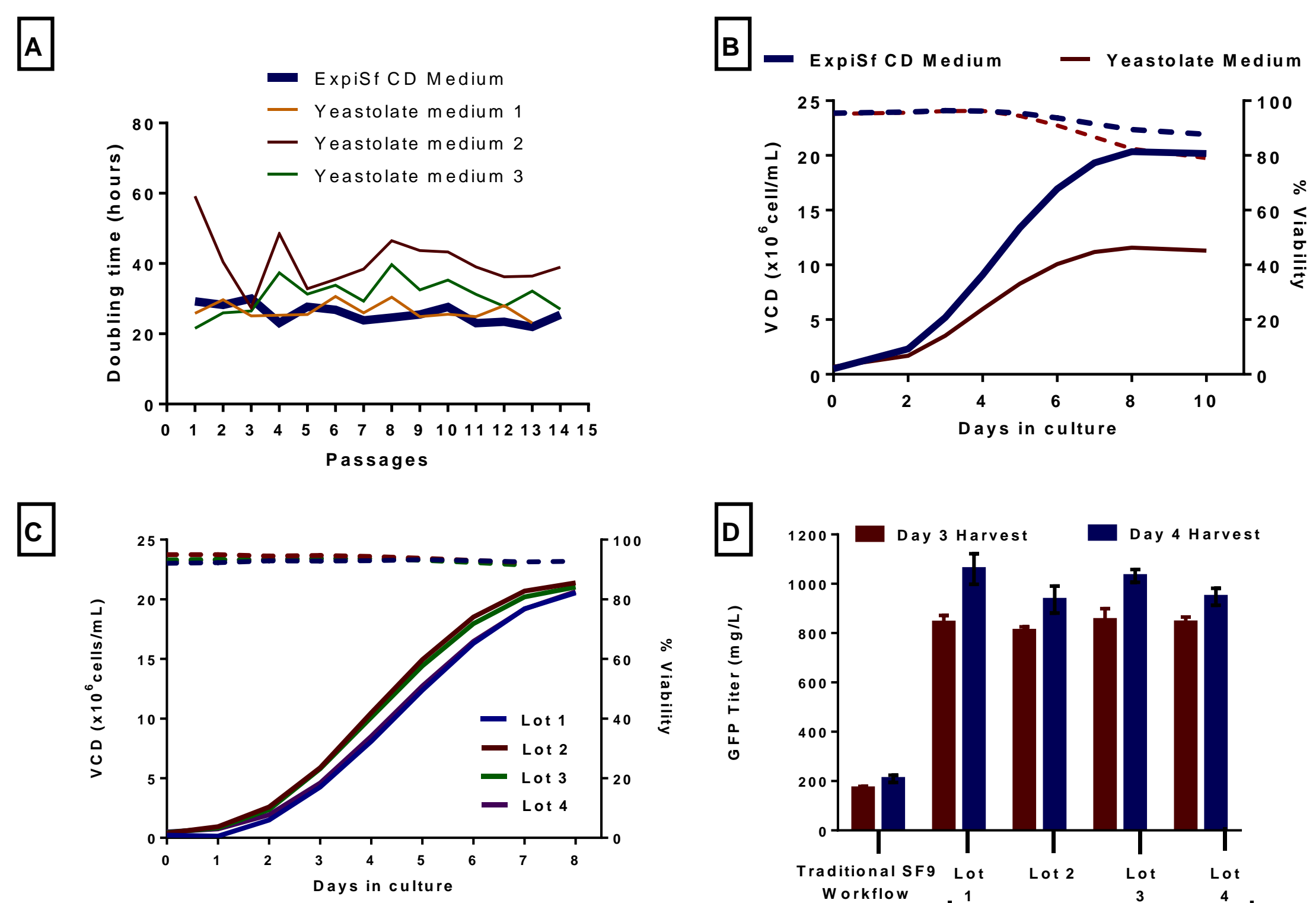


Figure 2. Characteristics of ExpiSf9 CD Medium

(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<sup>6</sup> 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. Growth of 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

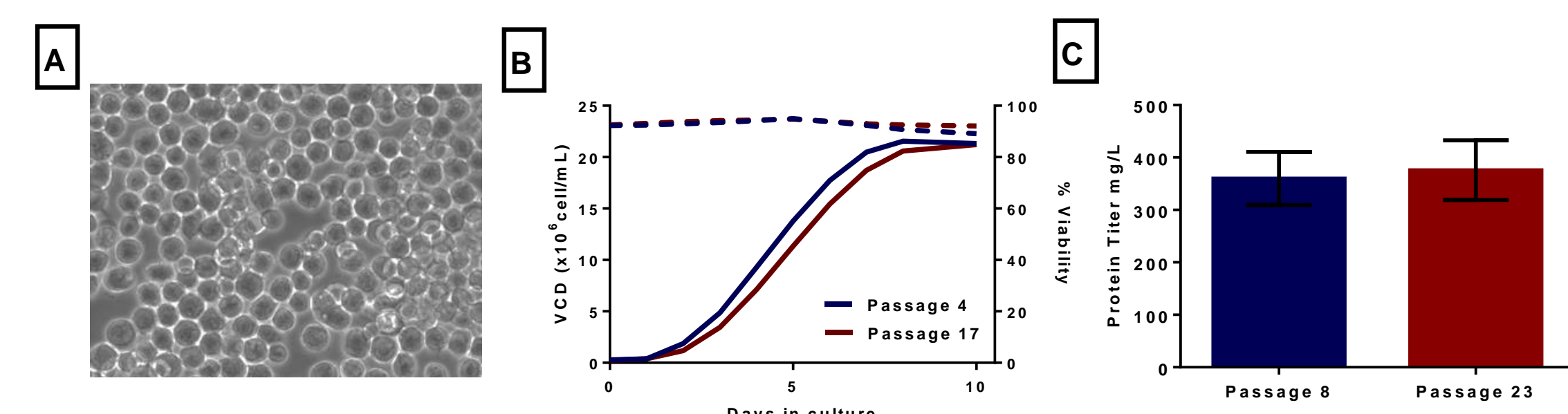
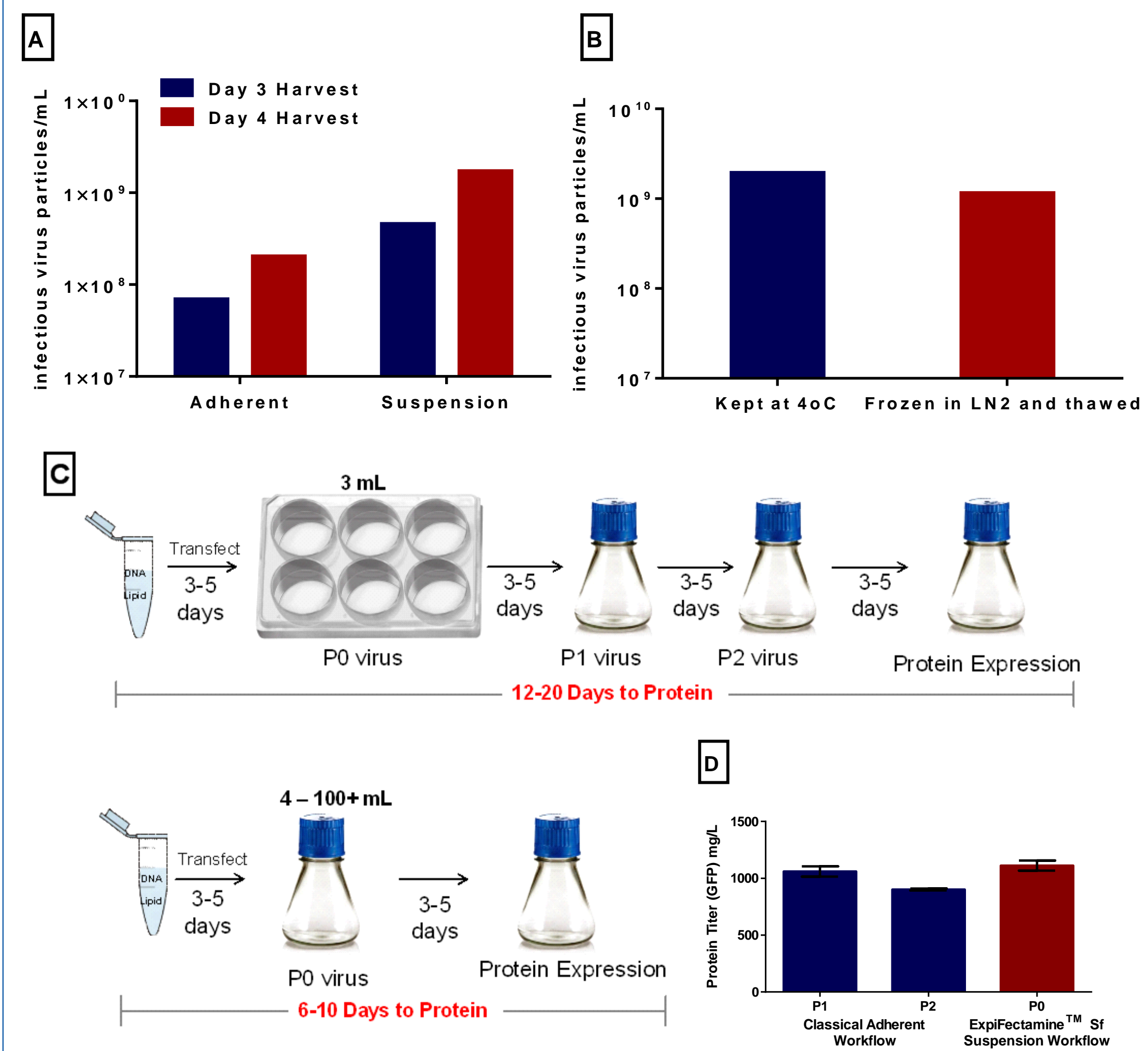


Figure 3. Characterization of Sf9 cells adapted to ExpiSf Chemically-Defined medium (A) ExpiSf9 cells morphology (B) Consistent growth over passages. Lines represents the growth of ExpiSf9 cells in ExpiSf CD Medium at passage 4 (Blue line) and passage 17 (Red Line) (C) Consistent Protein Expression over passages. Protein titers at passage 8 (Blue Bar) and passage 23 (Red Bar)

## III. Characterization of ExpiFectamine™ Sf Transfection Reagent

- ExpiFectamine™ Sf Transfection Reagent**
- Convenient protocol
  - Scalable virus production
    - Adherent - 6-well plate to T-25 flask format
    - Suspension - 4mL to 100mL or greater



## 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) Baculovirus 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 can be obtained 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 added 18-24hr before infection
- Optimized for ExpiSf CD Medium

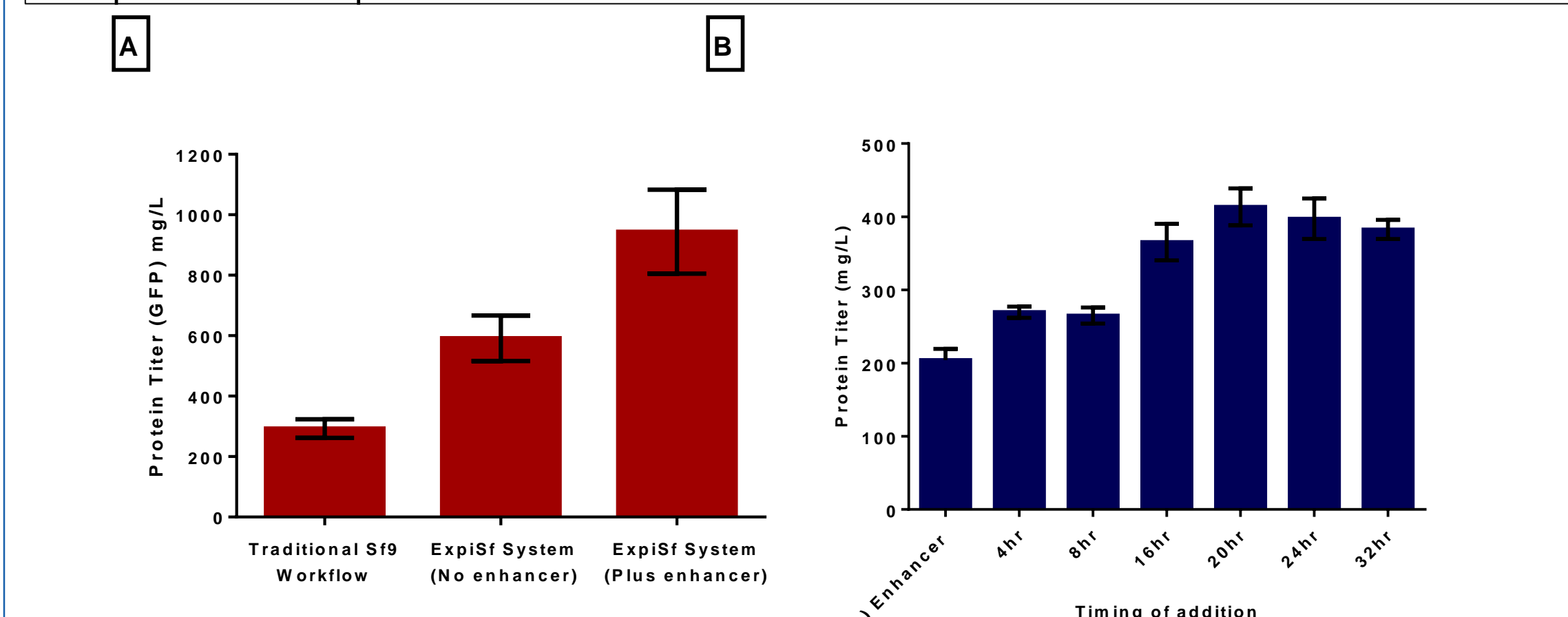


Figure 5. Characterization of ExpiSf™ Enhancer (A) ExpiSf Enhancer, used in conjunction with ExpiSf CD Medium and ExpiSf9 cells, generated 3-fold higher GFP titers than a traditional Sf9 workflow. ExpiSf Enhancer nearly doubled protein titers compared to the ExpiSf System without enhancer addition. (B) Addition of ExpiSf Enhancer 18-24hr prior to infection gives the highest protein titer improvement.

## V. Protein Expression Workflow

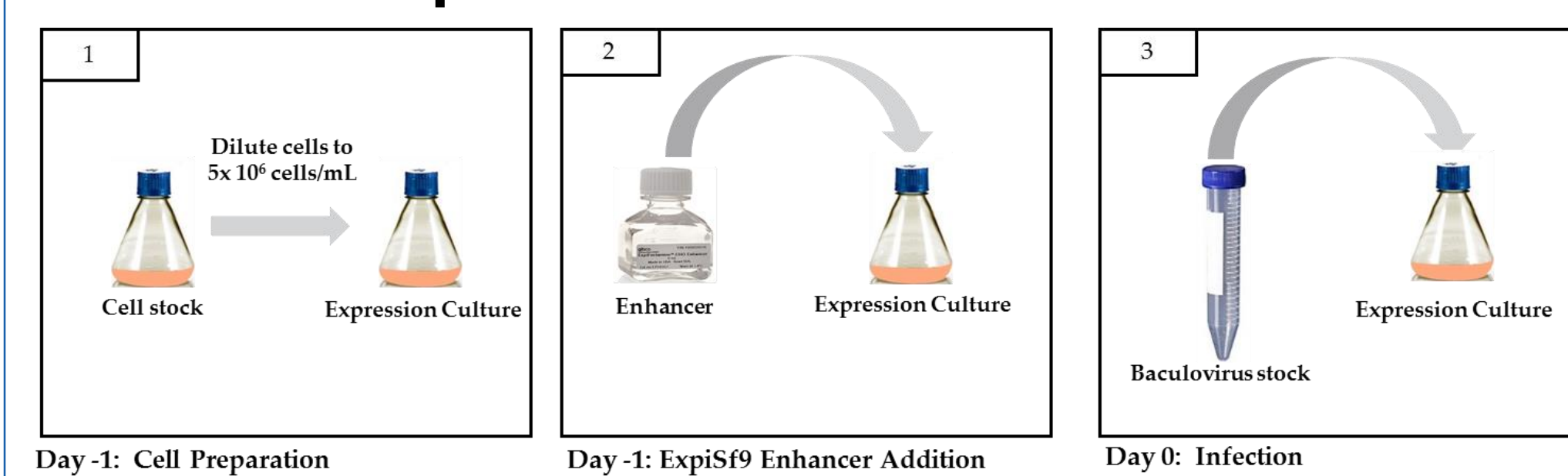


Figure 6. Protein Expression Workflow

## VI. Comparison of protein expression between ExpiSf System and High Five cells

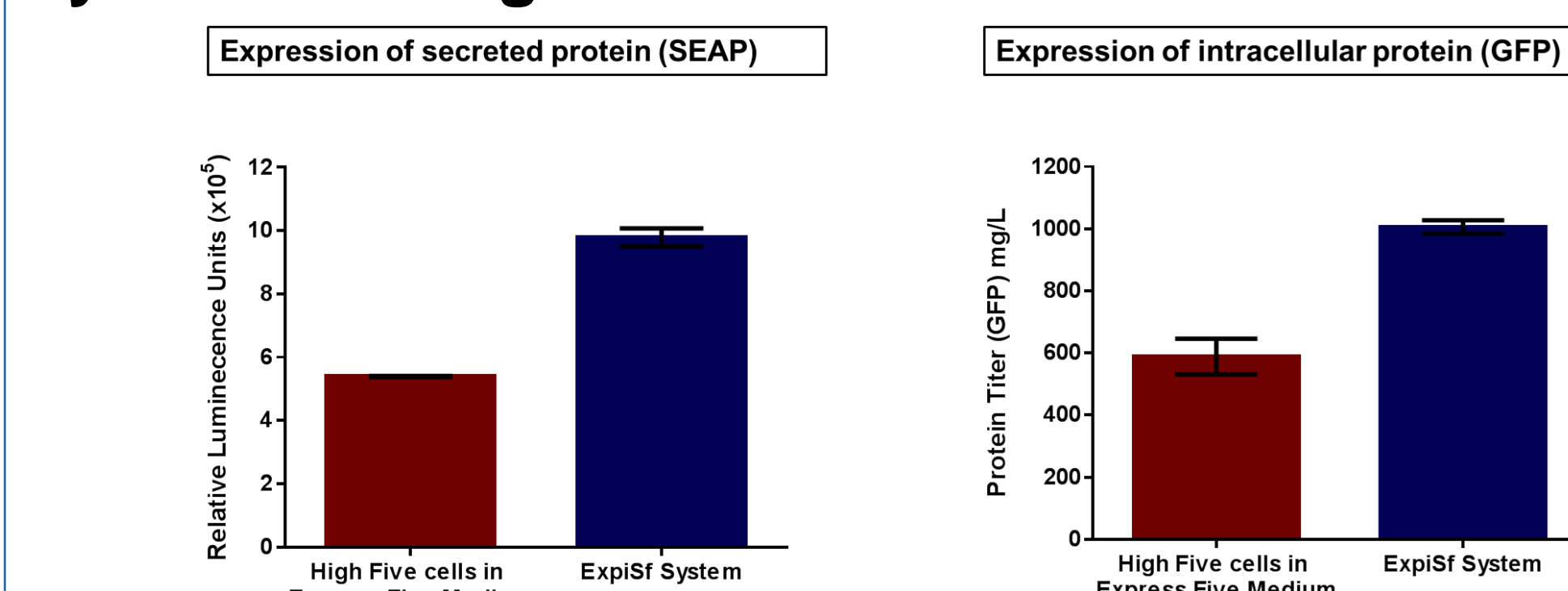


Figure 7. Protein expression in ExpiSf System and High Five cells (A) SEAP activity measured by chemiluminescence assay for protein expressed High Five cells in Expressive medium and in the ExpiSf System (B) GFP protein titers measured by fluorescent assay for protein expressed High Five cells in Expressive medium and in the ExpiSf System

## VII. ExpiSf System Scalability

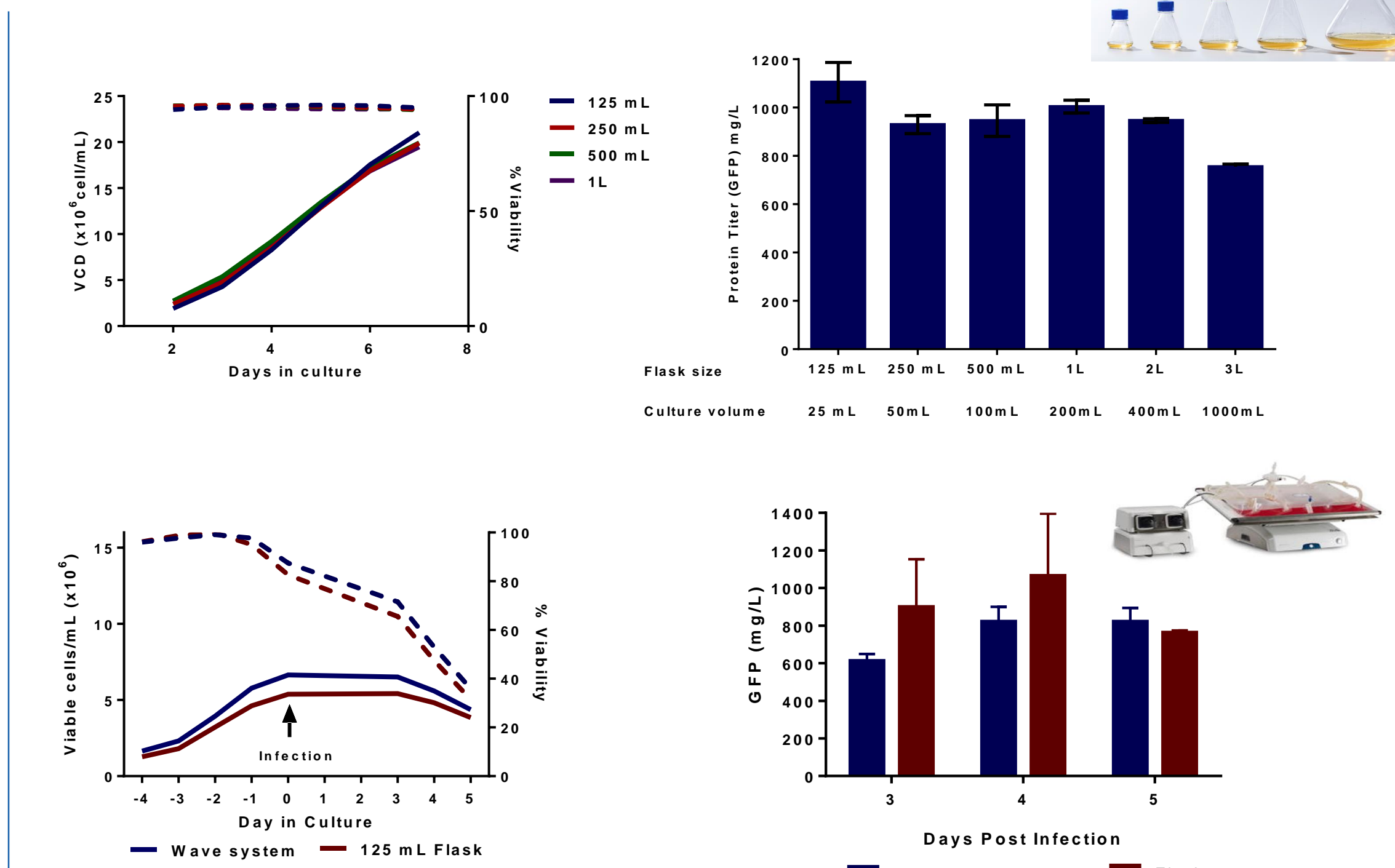


Figure 8. ExpiSf System can be scaled up or scaled down ExpiSf System is directly scalable from 125mL to 1L flask size. Comparable cell growth (A) and protein expression (B) were achieved at 125 rpm shake speed. The ExpiSf System also can be scaled down to 24 deep well plate for cell growth (C) and protein expression (D).

## VIII. Protein Expression in ExpiSf System vs Traditional Systems

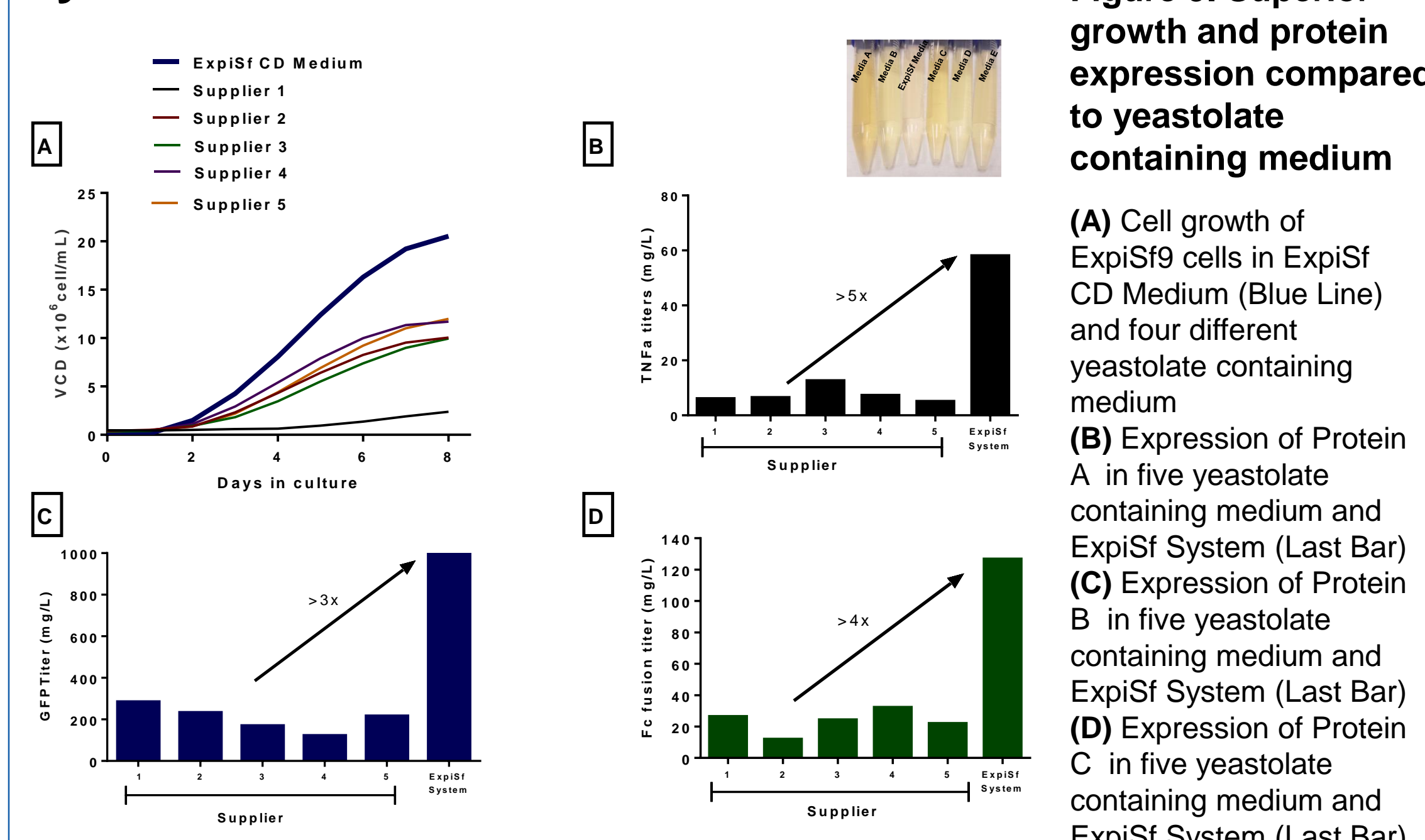


Figure 9. Superior growth and protein expression compared to yeastolate containing medium (A) Cell growth of ExpiSf9 cells in ExpiSf CD Medium (Blue Line) and four different yeastolate containing medium (B) Expression of Protein A in five yeastolate containing medium and ExpiSf System (Last Bar) (C) Expression of Protein B in five yeastolate containing medium and ExpiSf System (Last Bar) (D) Expression of Protein C in five yeastolate containing medium and ExpiSf System (Last Bar)

## IX. Protein Characterization in ExpiSf System

### Secreted Proteins

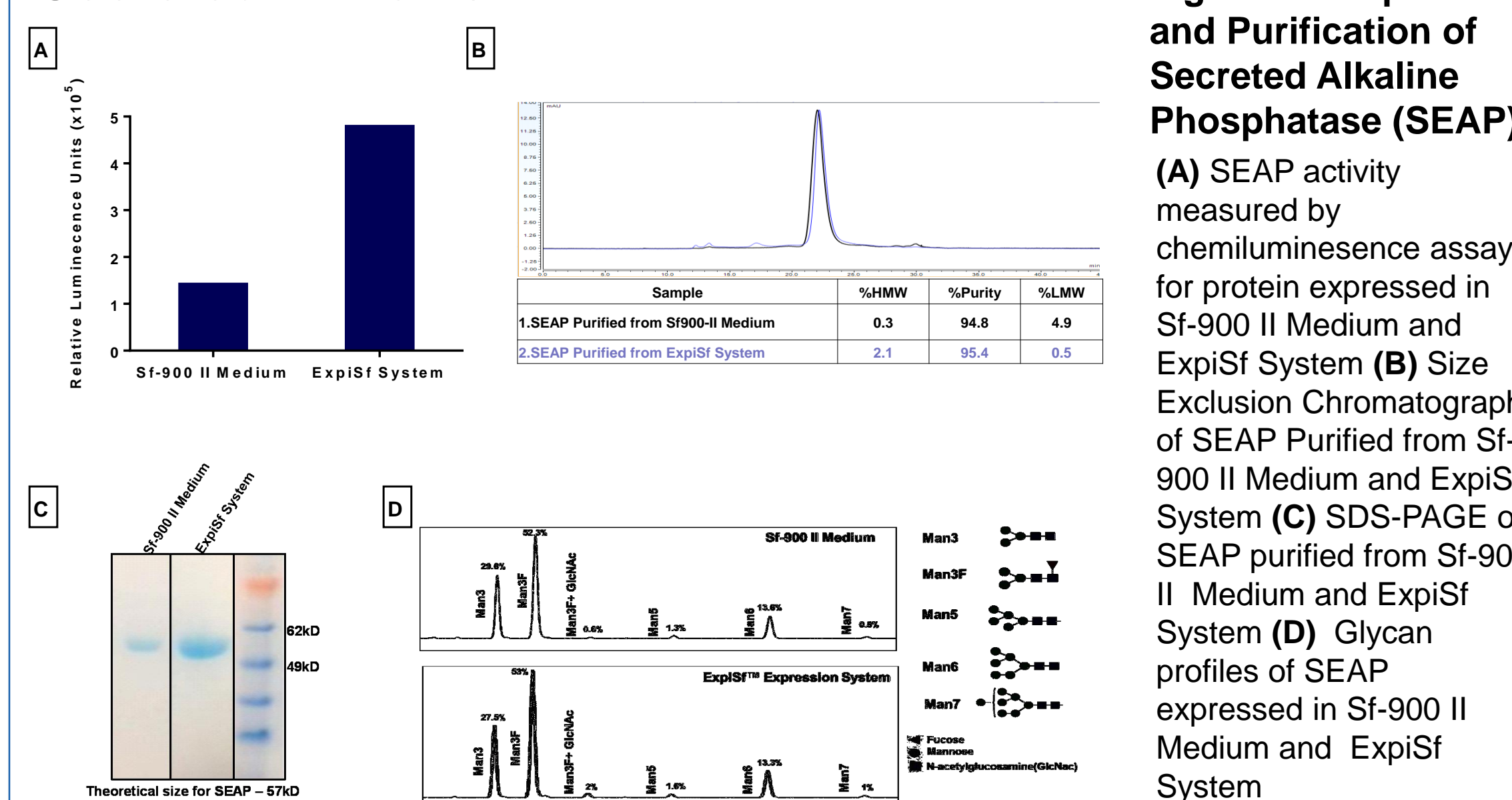


Figure 10. Expression and Purification of Secreted Alkaline Phosphatase (SEAP) (A) SEAP activity measured by chemiluminescence assay for protein expressed in Sf-900 II Medium and ExpiSf System (B) Size Exclusion Chromatograph of SEAP Purified from Sf-900 II Medium and ExpiSf System (C) SDS-PAGE of SEAP Purified from Sf-900 II Medium and ExpiSf System (D) Glycan profiles of SEAP expressed in Sf-900 II Medium and ExpiSf System

### G-protein coupled receptors

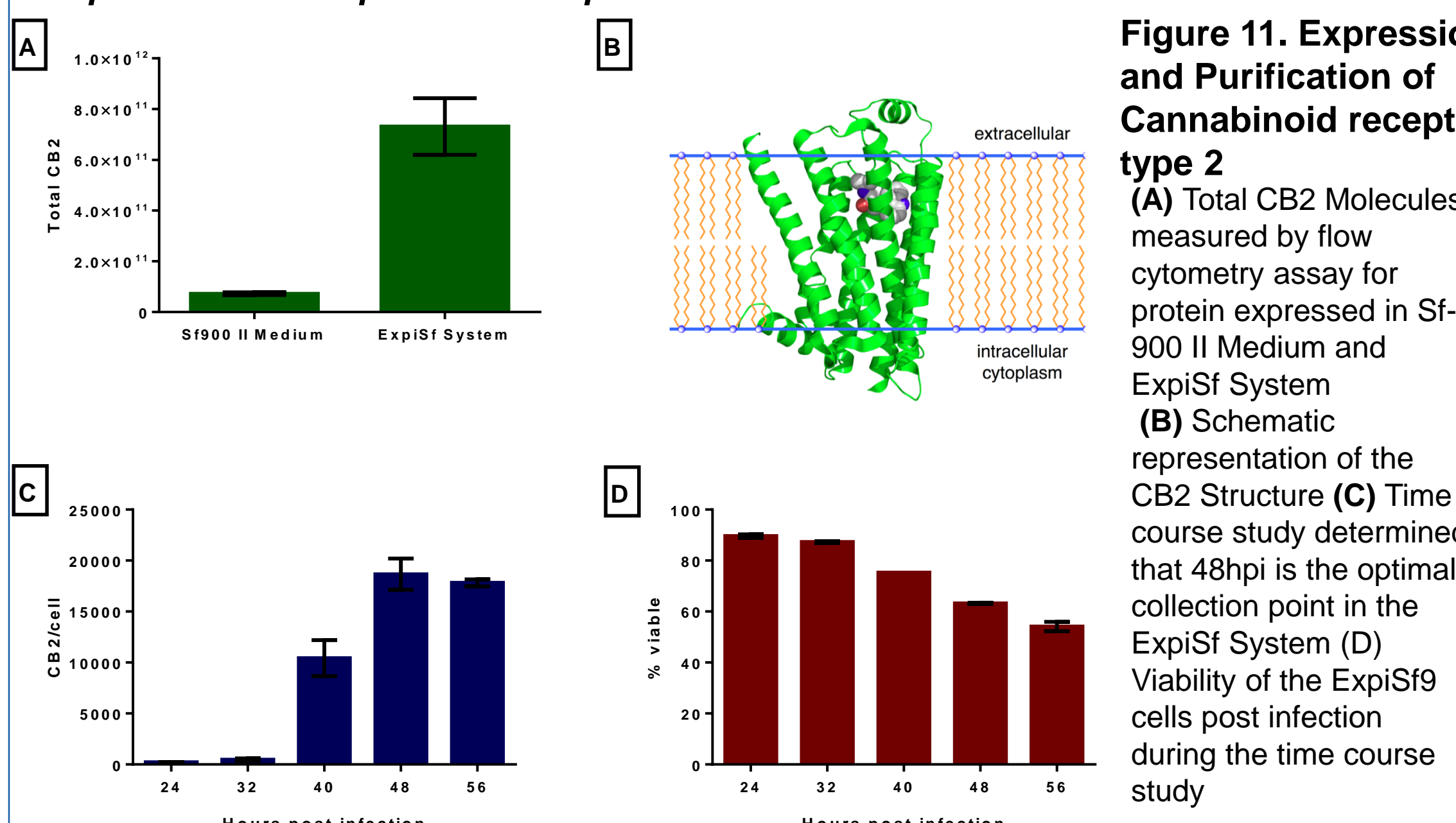


Figure 11. Expression and Purification of Cannabinoid receptor type 2 (A) Total CB2 Molecules measured by flow cytometry assay for protein expressed in Sf-900 II Medium and ExpiSf System (B) Schematic representation of the CB2 Structure (C) Time course study determined that 48hpi is the optimal collection point in the ExpiSf System (D) Viability of the ExpiSf9 cells post infection during the time course study

## CONCLUSIONS

We describe 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. This performance enhancement was made possible through the incorporation of multiple novel reagents, including: (1) Chemically Defined Culture Medium that allows for high density cell growth and infection, (2) Sf9 Cells Adapted to grow optimally in the CD Medium, (3) an optimized transfection reagent for baculovirus generation, (4) a novel pre-infection expression enhancer solution, and (6) a simple-to-perform workflow.