

An Optimised Transfection Platform for the *EpiCHO* Transient Expression System in Serum-free and Chemically Defined Media

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Introduction

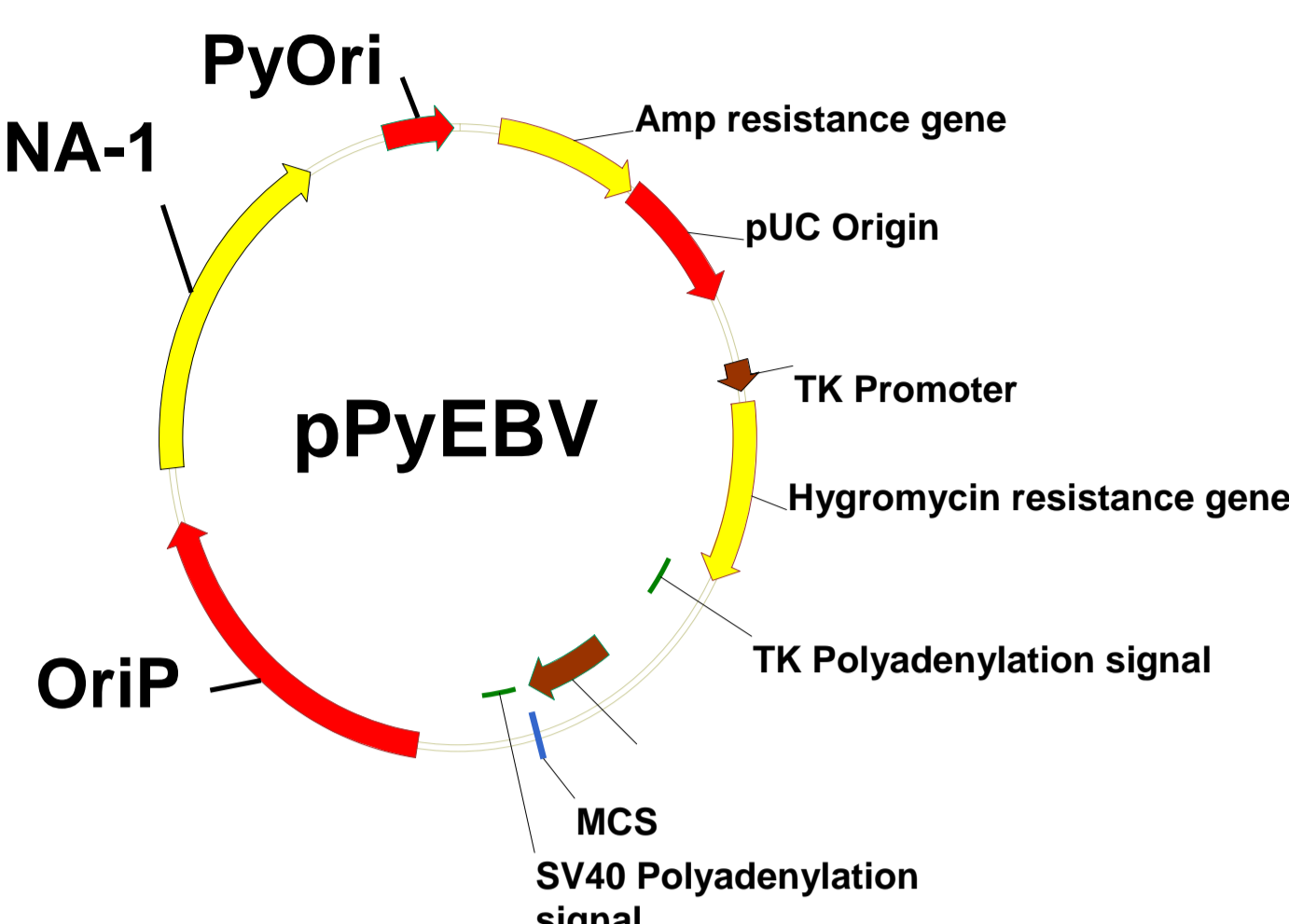
EpiCHO is a transient expression system that enables elevated and prolonged expression of recombinant proteins in CHO. It is comprised of two main components:

•CHO-T:

- A stable CHO cell line that constitutively expresses the Polyoma Virus (PyV) Large-T antigen (PyLT)

•pPyEBV:

- An expression vector which contains the PyV Origin of replication (*PyOri*), Epstein-Barr Virus (EBV) nuclear antigen-1 gene (EBNA-1) and EBV origin of replication (*oriP*)



- PyLT binds to *PyOri* to initiate plasmid replication

- EBNA-1 binds to *oriP* and promotes episomal maintenance during cell division

We have developed a serum-free, prolonged and scalable transient expression process using the *EpiCHO* system, achieving an elevated Mab concentration of 150 mg/L in 25 days. This is the highest recorded Mab concentration produced by CHO cells in a transient system

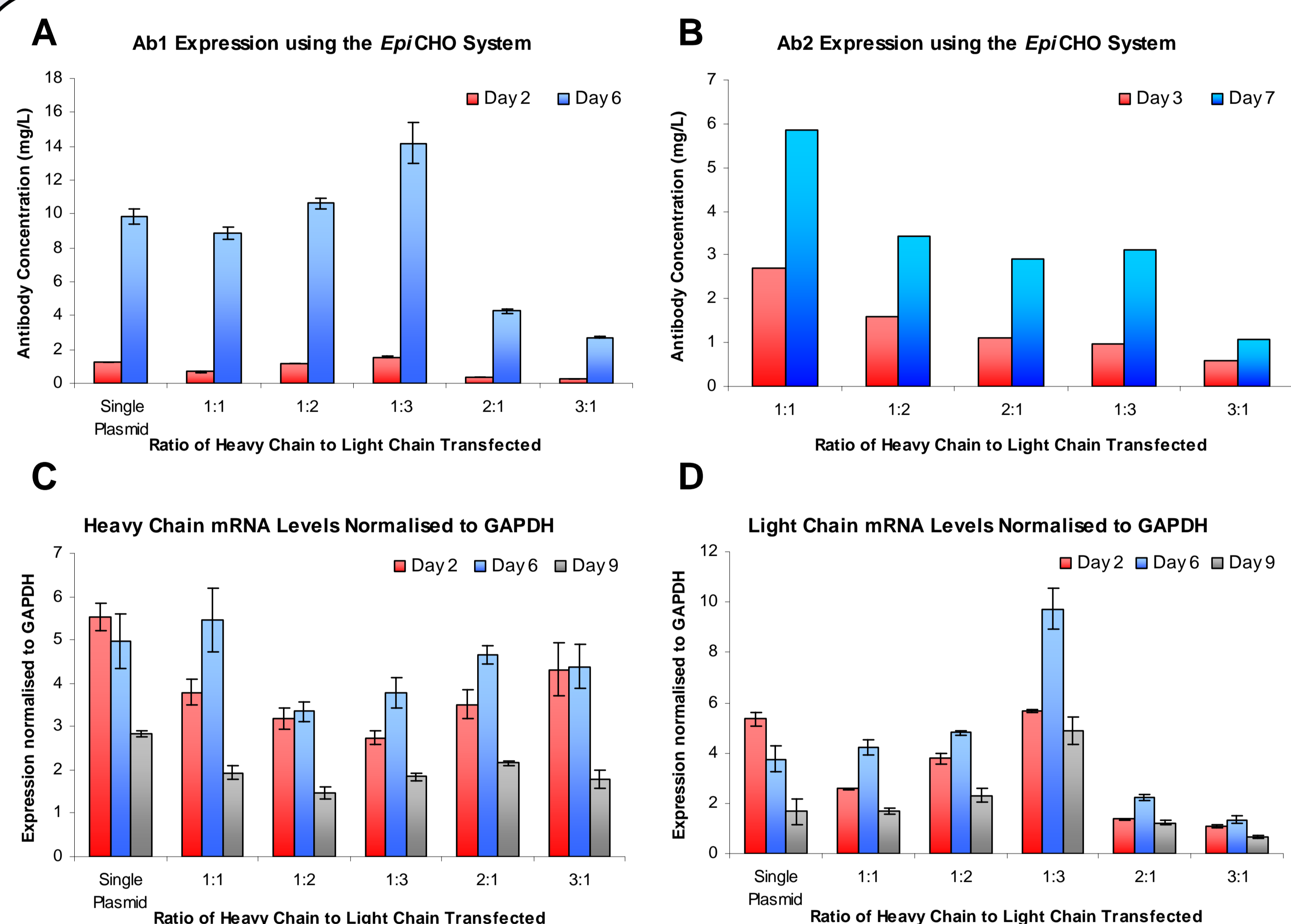


Figure 1: Enhancing Ab1 and Ab2 Mab expression with *EpiCHO* by optimising heavy chain to light chain plasmid DNA ratios for transfection

Suspension CHO-T cells were transfected with varying mass ratios of pPyEBV-Ab1 heavy chain, pPyEBV-Ab1 light chain or a single vector encoding both chains. A similar study was also performed for the Mab Ab2 (no single vector control).

Expression was monitored by ELISA and qRT-PCR (Ab1 only) for 7 to 9 days post-transfection. Ab1 (A) and Ab2 concentrations (B) indicate that a heavy to light chain ratio of 1:3 and 1:1 respectively attain the highest yields for each Mab. Ab1 heavy (C) and light chain (D) mRNA levels also highlight this, with the latter significantly higher for the ratio of 1:3 at days 6 and 9. (Error bars: Represent the standard deviation of biological triplicates)

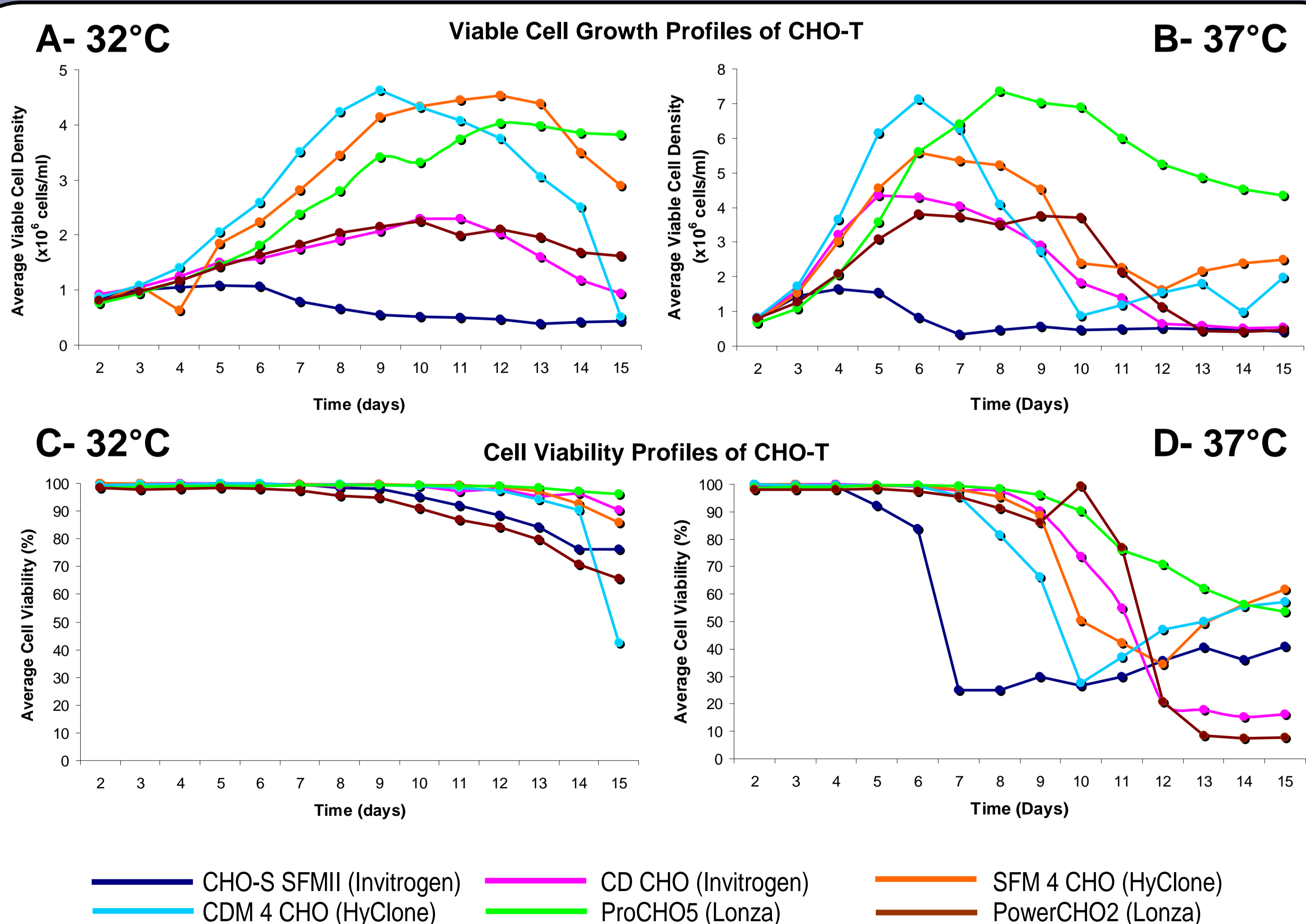


Figure 2: Evaluation of Growth Media Leads to the Improved Growth of CHO-T

Suspension CHO-T cells were adapted to grow in various chemically defined and serum-free media, with growth and viability profiles analysed at 32 and 37°C (in duplicate). Cells were seeded at 0.75x10⁶ cells/ml (to mimic the conditions of a post-transfection dilution) and grown in batch for 15 days.

Improved growth compared to the CHO-S SFMII control at 32 (A) and 37°C (B) is evident, with a maximum cell density of ~7.5x10⁶ cells/ml attained by day 8 with ProCHO5 at 37 °C. Cell viability is also prolonged with all media when compared to CHO-S SFMII.

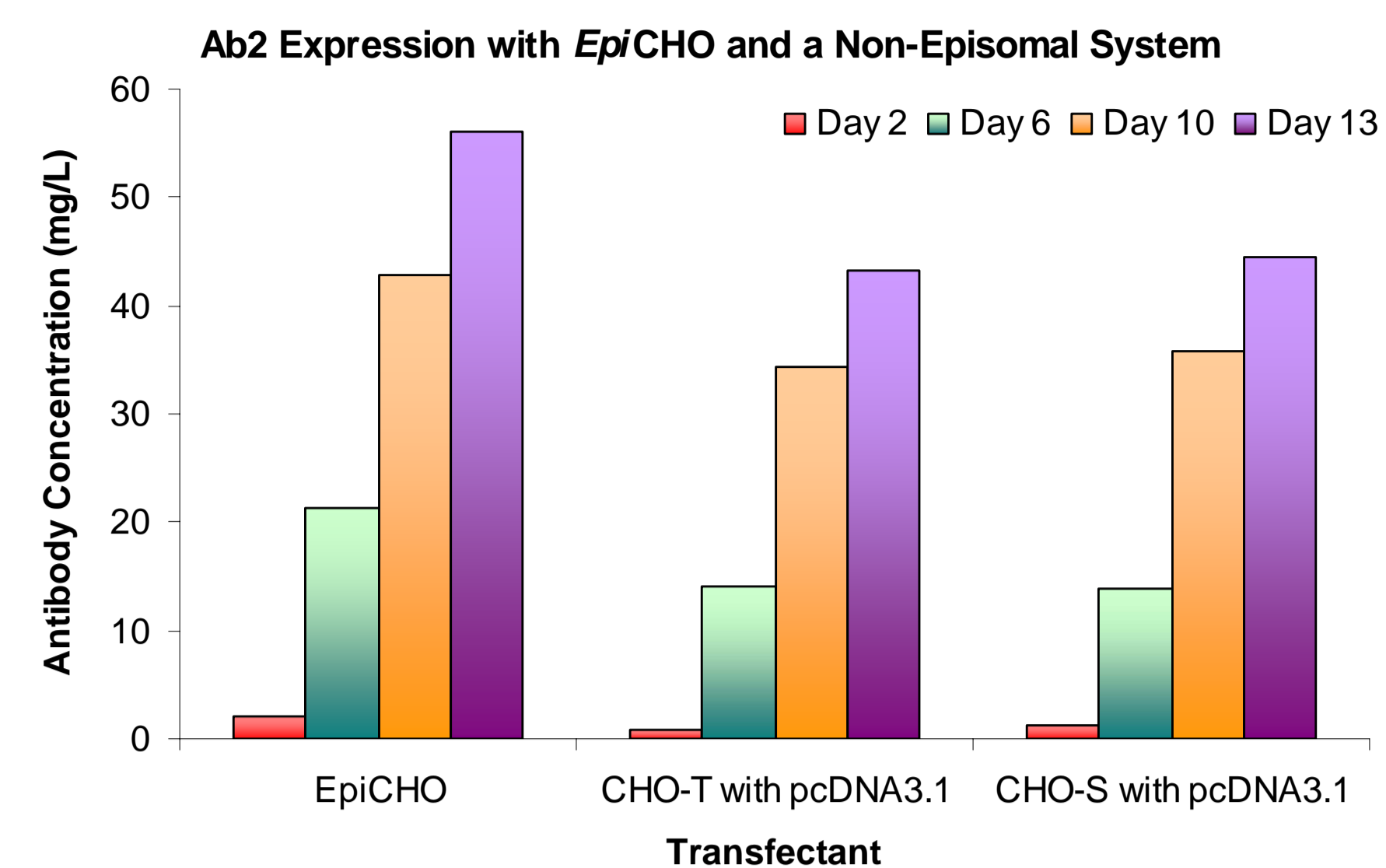


Figure 3: Elevated Mab expression with *EpiCHO* compared to a non-episomal system

Suspension CHO-T cells growing in CHO-S SFMII were co-transfected (using Lipofectamine 2000) with pPyEBV-Ab2 heavy chain and pPyEBV-Ab2 light chain at a mass-ratio of 1:1 in 50 ml (in duplicate). Similarly, the same CHO-T cells and CHO-S cells growing in CD CHO were co-transfected with pcDNA3.1-Ab2 heavy chain and pcDNA3.1-Ab2 light chain using PEI-Max. Ab2 expression over 13 days was monitored by ELISA.

CHO-T cells transfected with pPyEBV produced ~55 mg/L Mab after 13 days, compared with ~42 mg/L obtained with CHO-T and CHO-S cells transfected with a non-episomal vector. This highlights the improvements that are made with transient protein expression through the replication and retention of transfected plasmid DNA.

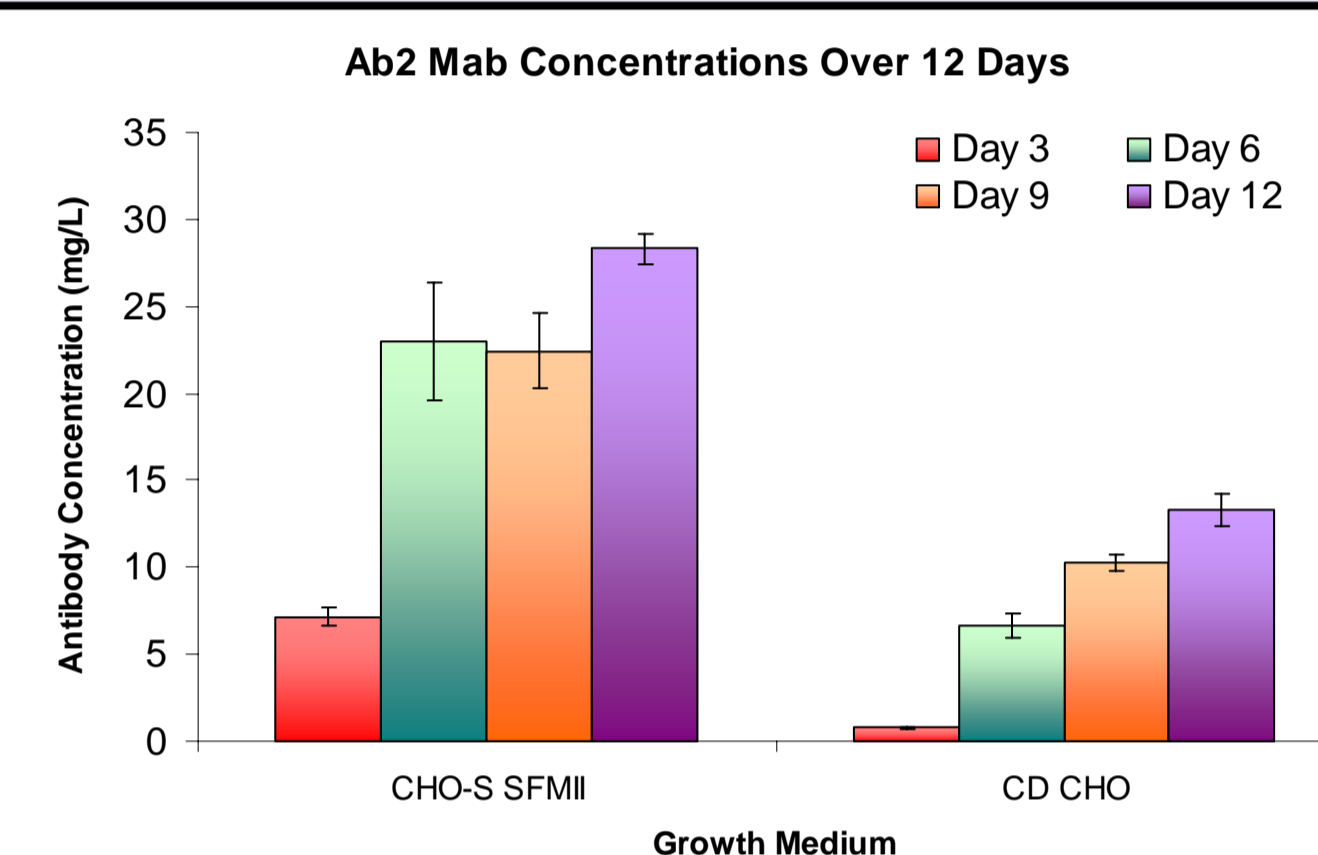


Figure 4: Ab2 Mab expression with the *EpiCHO* system pre-optimisation

Suspension CHO-T cells adapted to grow in CHO-S SFMII and CD CHO were co-transfected with pPyEBV-Ab2 heavy chain and pPyEBV-Ab2 light chain at a mass-ratio of 1:1 in 50 ml. Ab2 expression over 12 days was monitored by ELISA.

Cells in CHO-S SFMII (transfected using Lipofectamine 2000) obtained ~30 mg/L Mab after 12 days. Cells in CD CHO (transfected using PEI-Max) obtained ~15 mg/L of Mab after 12 days. (Error bars: Represent standard deviation of biological triplicates)

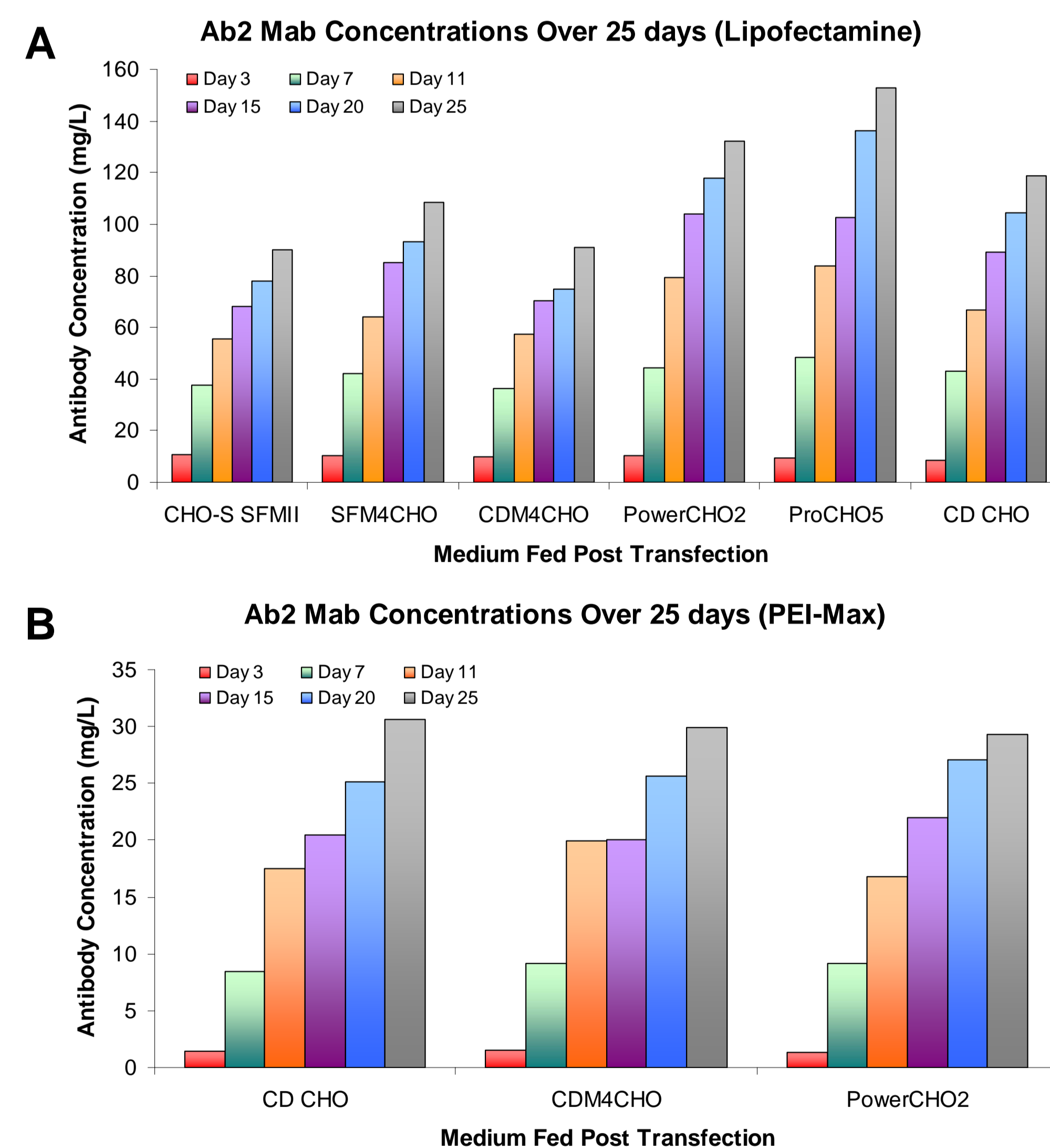


Figure 5: 150 mg/L of Ab2 Mab produced using the *EpiCHO* system after 25 days

Suspension CHO-T cells adapted to grow in CHO-S SFMII and CD CHO were co-transfected with pPyEBV-Ab2 heavy chain and pPyEBV-Ab2 light chain at a mass-ratio of 1:1 in 50 ml (in duplicate). Various media and IGF-1 were supplemented to cells 4 hours post-transfection, with expression monitored over 25 days by ELISA.

All CHO-S SFMII variants (transfected using Lipofectamine 2000) produced elevated levels of Ab2, with ProCHO5 supplemented cells attaining concentrations of ~150 mg/L (A) after 25 days. All CD CHO variants (transfected using PEI-Max) also produced elevated levels of Ab2, with CD CHO supplemented cells attaining concentrations of ~30 mg/L (B).

Conclusions

- *EpiCHO* is a transient expression system designed to promote elevated and prolonged recombinant protein expression.
- The combination of plasmid replication and retention in the *EpiCHO* system provides for significant improvements in recombinant protein expression.
- Through the optimisation of transfection and examination of various growth media, we have obtained Mab concentrations of 150 mg/L and prolonged expression for up to 25 days. This is the highest recorded Mab concentration produced by CHO cells in a transient system.
- *EpiCHO* provides a platform for the rapid scalable production of therapeutic proteins for early stage development.