

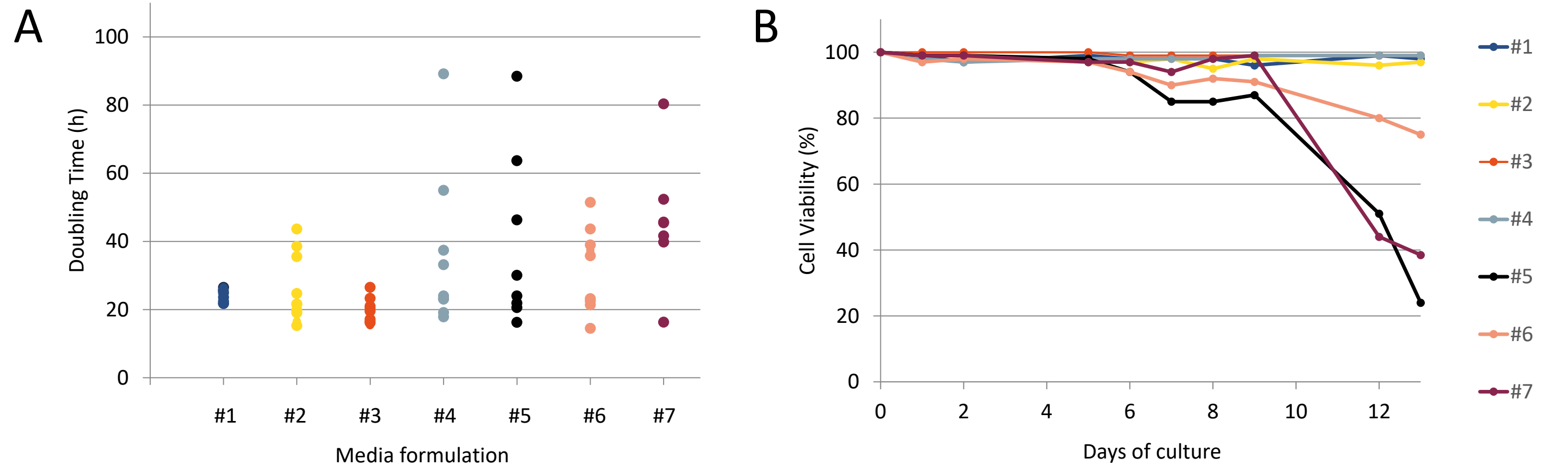
Development and Optimization of an Advanced Transient Expression System in CHO Cells

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Abstract

The growing need in the biopharmaceutical market for various recombinant proteins and antibodies produced in a short time requires an efficient and flexible system. Transient expression is a commonly used process to face this demand but is unfortunately limited by transfection efficiency and inherent productivity, especially with CHO cells. To overcome this issue, we developed an advanced transient expression system consisting in the synergistic association of a novel CHO chemically defined medium and a powerful transfection reagent. First, we show that this innovative medium allows easy cultivation of various strains of CHO cells without the need of an extensive sequential adaptation. In a second time, through the protocol optimization of a technologically advanced transfection solution, we demonstrate a major increase in our recombinant proteins yields.

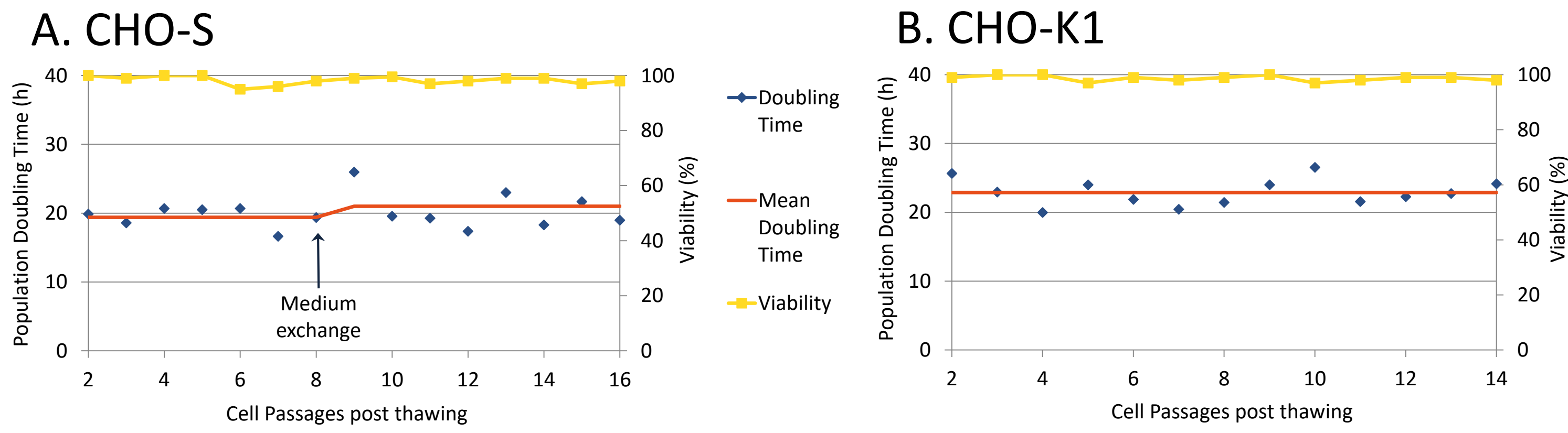
CHO cell Medium Optimization: FectoCHO™ CD Expression Medium



Several media formulation were tested for CHO-S cell culture to identify the optimal formulation for constant doubling time and sustained viability.

CHO-S cells were cultivated for 14 days in different media formulation. Doubling time (A) and cell viability (B) were measured at each passage using Trypan Blue exclusion. Medium #3 was chosen and is referred to as FectoCHO™ CD Expression Medium in this poster.

Direct and Effortless Adaptation of CHO cells in FectoCHO™ CD Expression Medium



FectoCHO™ CD Expression Medium allows direct adaptation and shows comparable growth performance for CHO-S, CHO-K1 and ExpiCHO™-S cells.

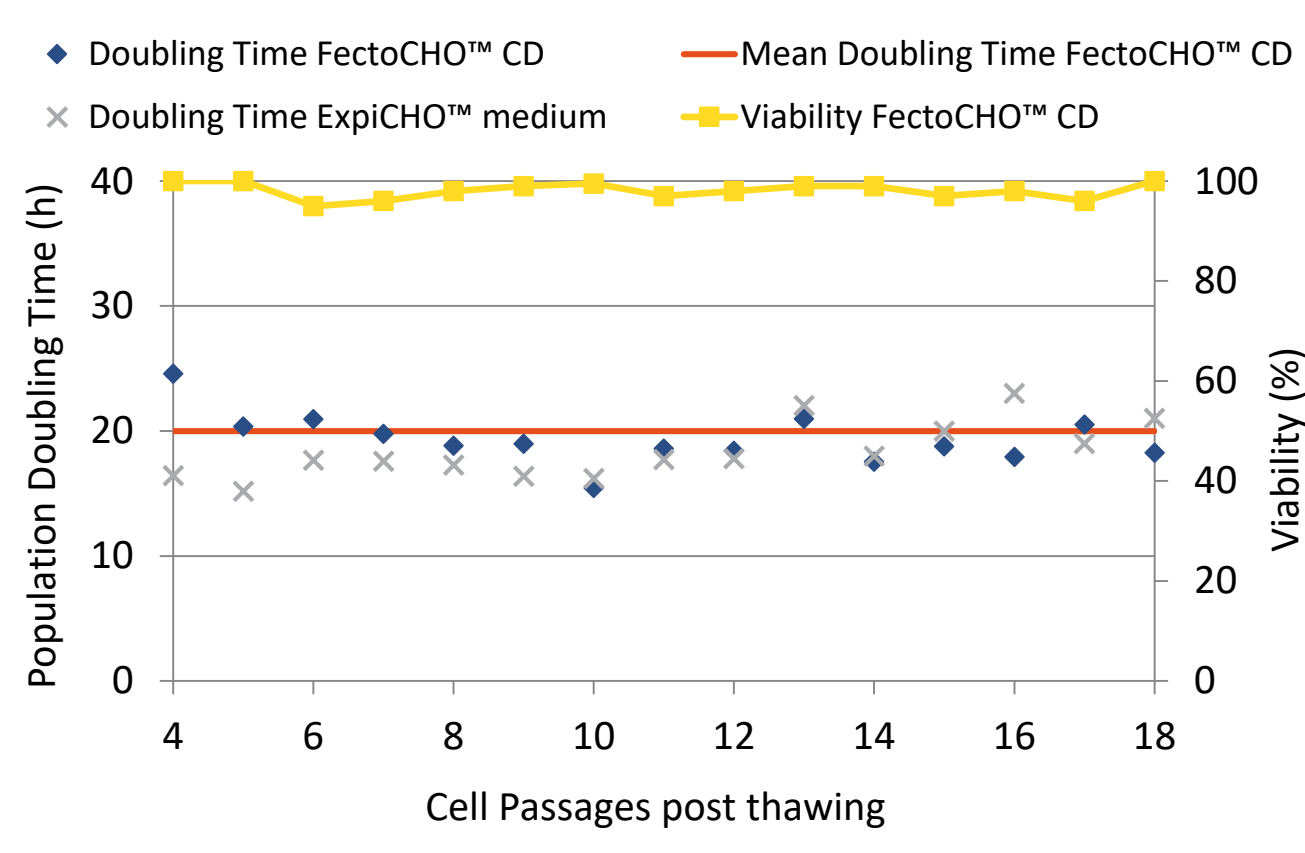
A. CHO-S cells were thawed and maintained in FreeStyle™ CHO Expression Medium. When reaching passage 8, cells were spun down, resuspended and maintained in 100% FectoCHO™ CD Expression Medium.

B. Suspension-adapted CHO-K1 were directly thawed and maintained in 100% FectoCHO™ CD Expression Medium.

C. ExpiCHO™-S cells were thawed and maintained either in FectoCHO™ CD Expression Medium or in ExpiCHO™ Expression Medium.

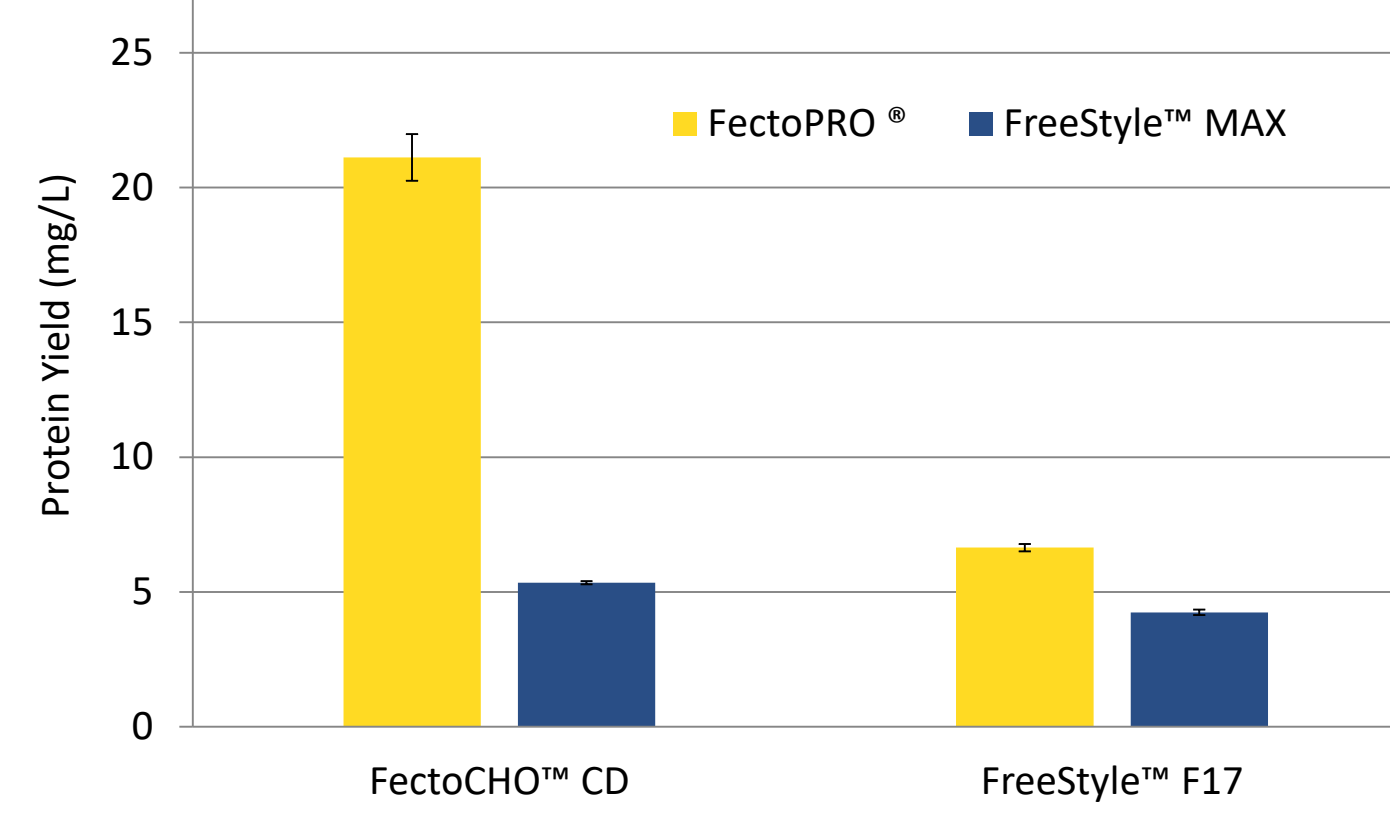
Cell densities and viabilities were measured using Trypan Blue exclusion.

C. ExpiCHO™-S

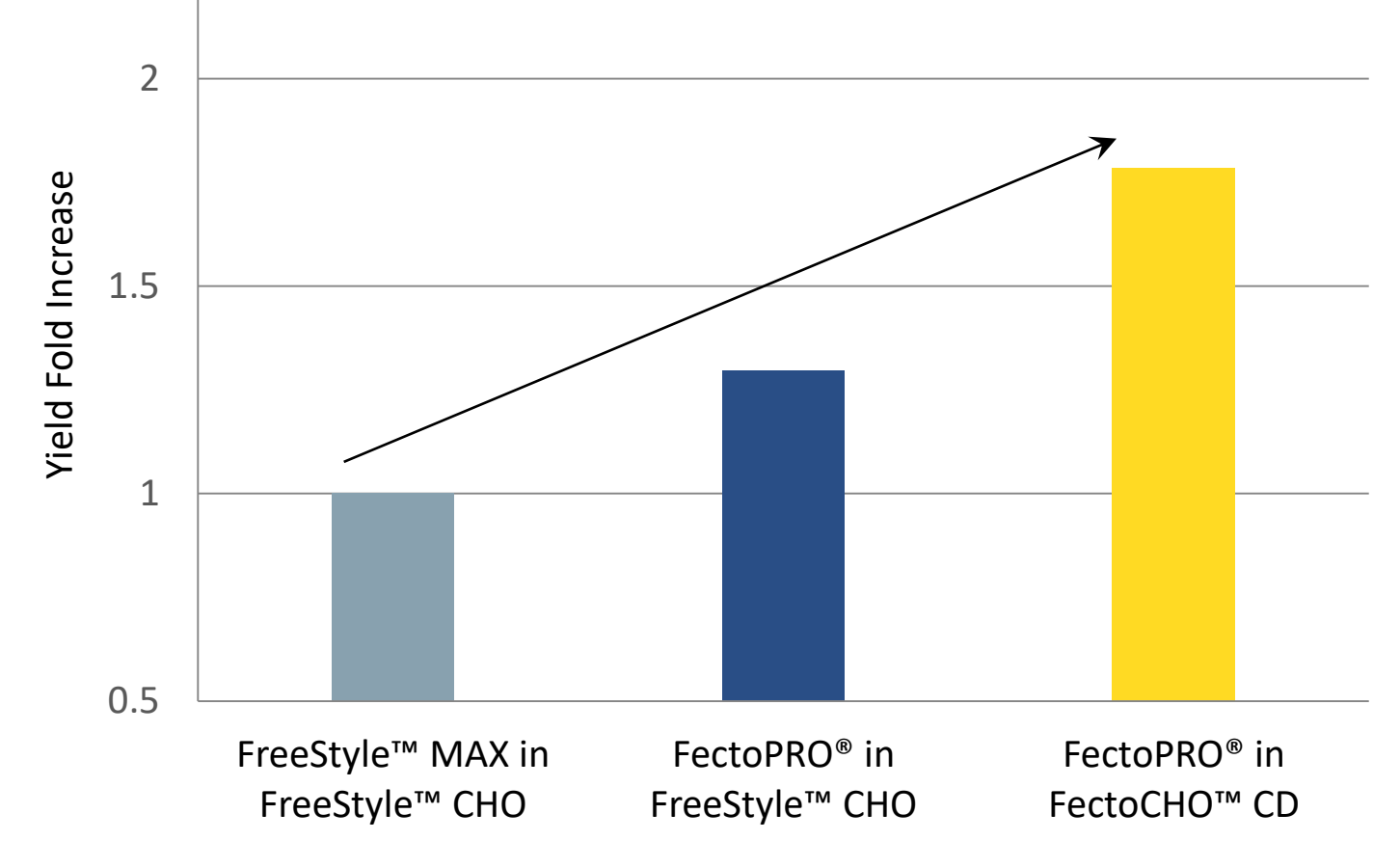


Increased Protein Productivity with FectoCHO™ Expression System

A. CHO-K1



B. CHO-S

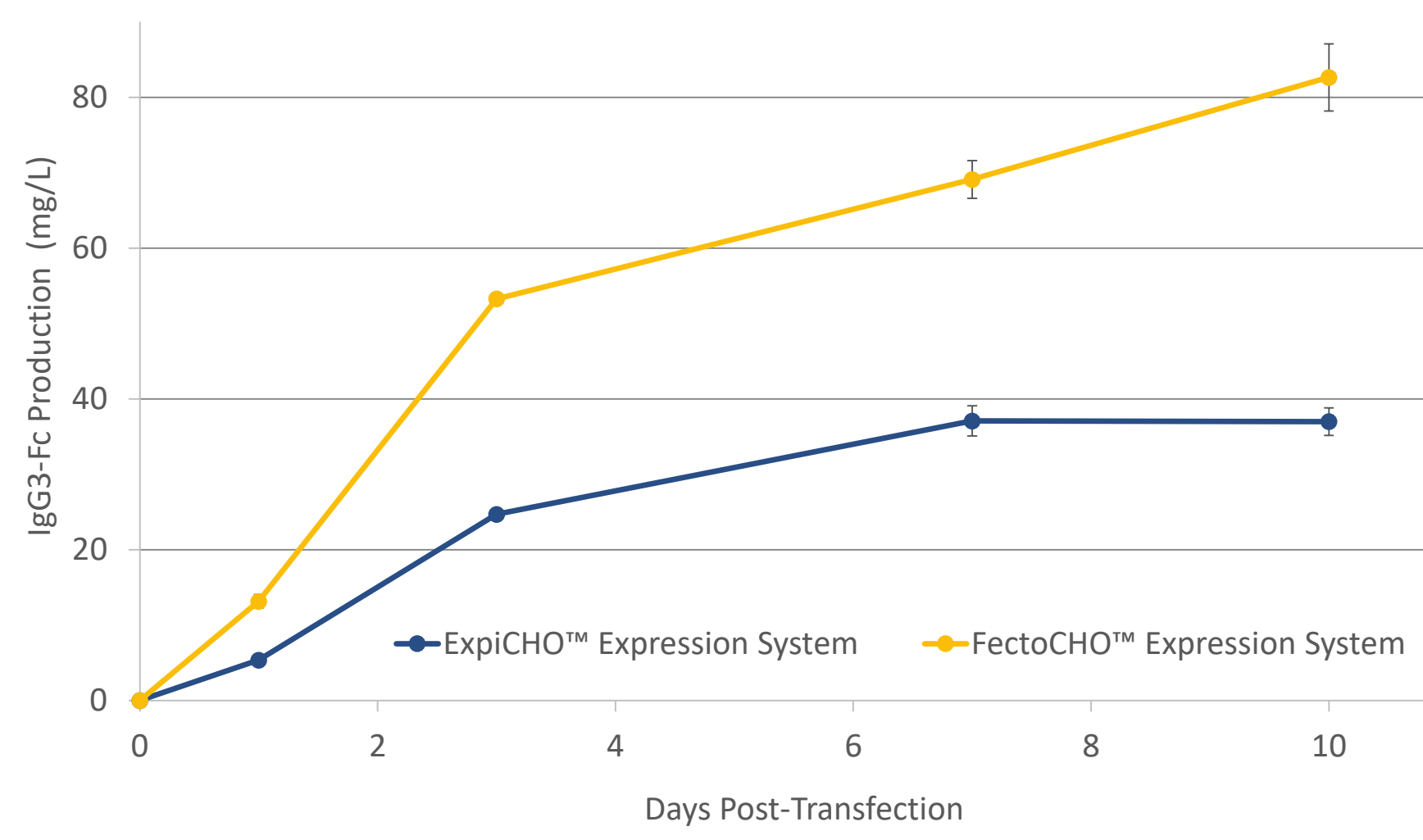


FectoCHO™ Expression System greatly increases productivity in comparison to competitors transfection reagents and expression media.

A. Suspension-adapted CHO-K1 cells were cultivated either in FectoCHO™ CD Expression medium or in FreeStyle™ F17, and transfected with FectoPRO® (1 µg DNA/mL) or FreeStyle™ MAX (1.25 µg DNA/mL) following the recommended protocols.

B. CHO-S cells were cultivated either in FectoCHO™ CD or in FreeStyle™ CHO, and transfected with FectoPRO® (1 µg DNA/mL) or FreeStyle™ MAX (1.25 µg DNA/mL) following the recommended protocols

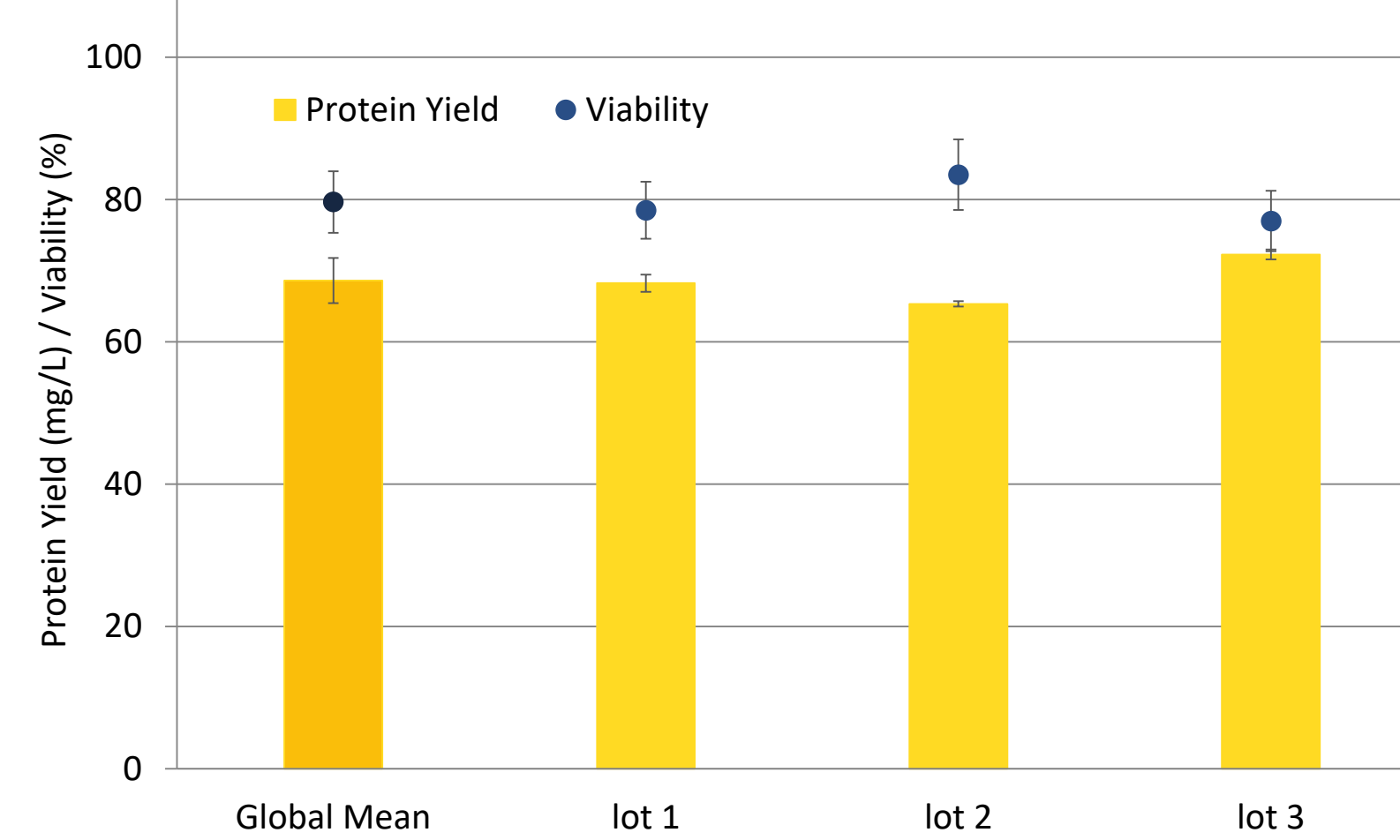
IgG₃-Fc production was assayed at day 7 post-transfection using protein G Biosensors (fortéBIO® BLItz).



Sustained productivity and high expression yield with ExpiCHO™-S cells in FectoCHO™ Expression System.

ExpiCHO™-S cells were either cultivated in FectoCHO™ CD Expression Medium and transfected with FectoPRO® following the recommended protocol, or in ExpiCHO™ Expression Medium and transfected following the recommended protocol with ExpiFectamine™ CHO transfection reagent. IgG₃-Fc production was assayed over 10 days post-transfection using protein G Biosensors (fortéBIO® BLItz).

Reproducibility and Adaptability of FectoCHO™ Expression System

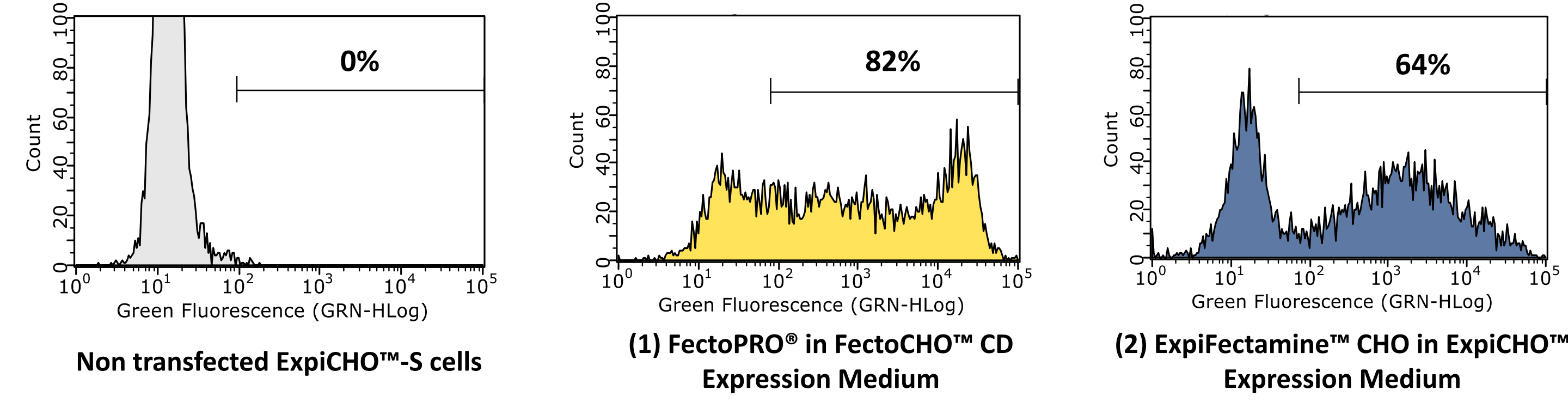


FectoCHO™ Expression System demonstrates a great lot to lot reproducibility

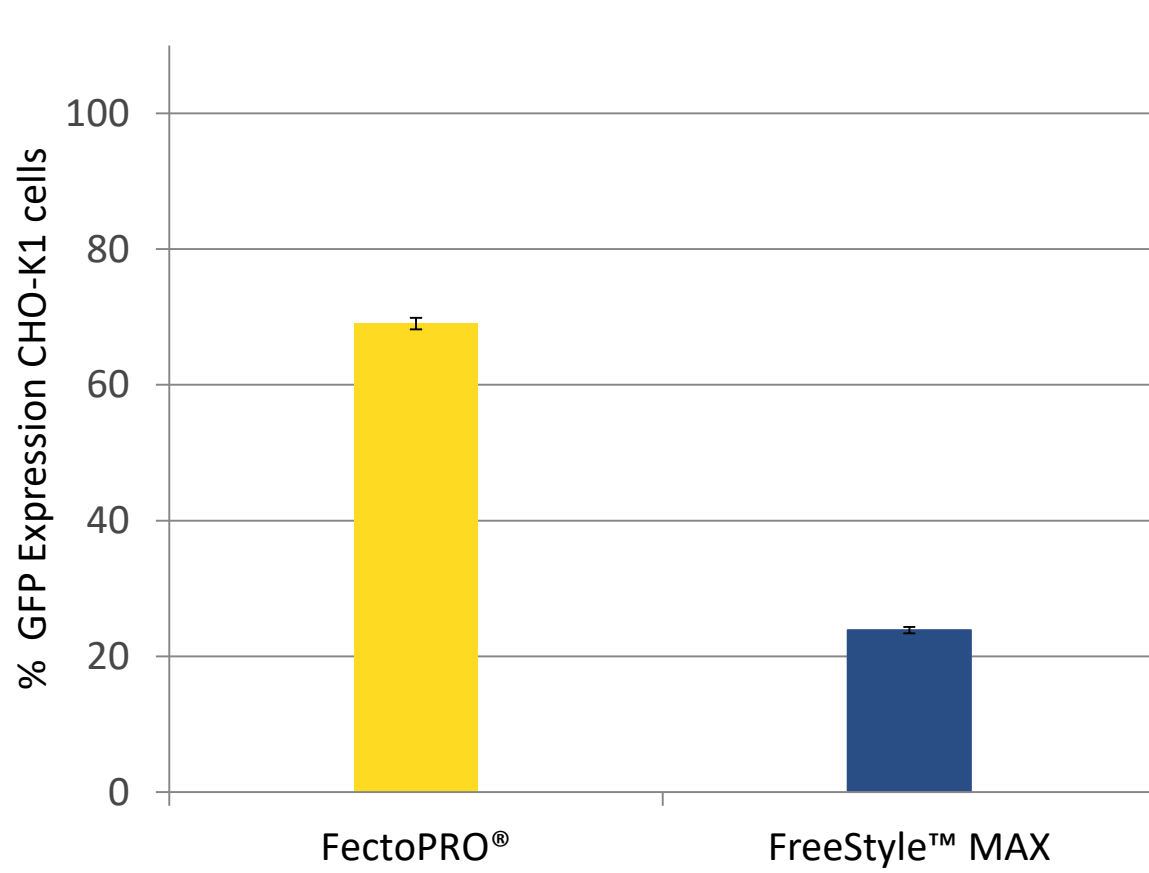
ExpiCHO™-S cells were cultivated in different FectoCHO™ CD Expression Medium lots and transfected with FectoPRO® following the recommended protocol. 7 days post-transfection, cell viability was measured using Trypan Blue exclusion. IgG₃-Fc production was assayed using protein G Biosensors (fortéBIO® BLItz).

Remarkable Transfection Efficiency

A. ExpiCHO™-S



B. CHO-K1

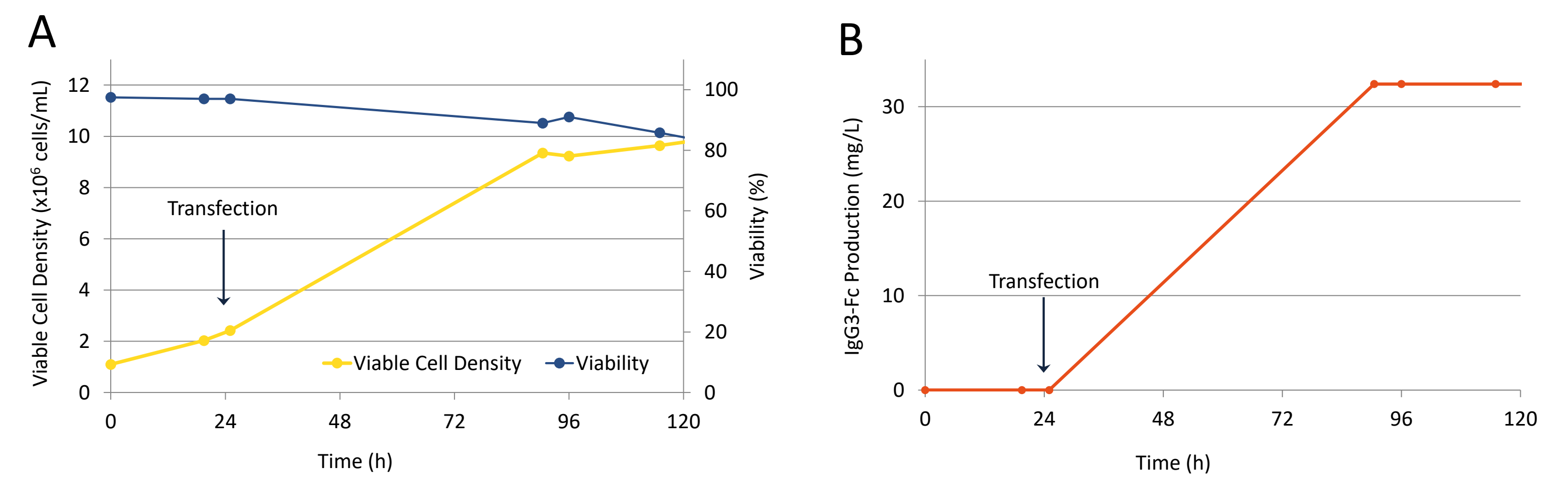


CHO cells cultivated in FectoCHO™ CD Expression Medium and transfected with FectoPRO® show superior transfection efficiencies.

A. ExpiCHO™-S cells were either cultivated in (1) FectoCHO™ CD Expression Medium and transfected with FectoPRO® following the recommended protocol, or in (2) ExpiCHO™ Expression Medium and transfected following the recommended protocol with ExpiFectamine™ CHO transfection reagent.

B. Suspension-adapted CHO-K1 cells were cultivated in FectoCHO™ CD Expression Medium and transfected with FectoPRO® (1 µg DNA/mL) or FreeStyle™ MAX (1.25 µg DNA/mL) following the recommended protocols.

Transfection efficiency (% GFP) was determined by flow capillary cytometry 24 hours post-transfection.



FectoCHO™ Expression System is perfectly compatible with cultivation and transient protein expression in bioreactor conditions and gives amazing protein yields.

CHO-S cells were seeded the day before transfection at 1x10⁶ cells/mL in a Mobius® single-use 3 L bioreactor (Merck) with 1.5 L of FectoCHO™ CD Expression Medium. The culture parameters were set as follows : 130 rpm, pH 7.1, 37°C, 40% pO₂. 24 hours after seeding, cells were transfected with FectoPRO® (0.8 µg DNA/mL) following the recommended protocol. (A) Cell viability and density were monitored regularly using Trypan Blue exclusion. (B) IgG₃-Fc production was assayed regularly using protein G Biosensors (fortéBIO® BLItz).

Conclusion

- FectoCHO™ CD is optimized for a direct adaptation and cultivation of CHO-S, CHO-K1 and ExpiCHO™-S cells
- FectoCHO™ CD allows optimal cell growth with a constant doubling time and a sustained viability
- FectoPRO® together with FectoCHO™ CD Expression Medium greatly increase protein productivity in CHO cells

- Easily adaptable from shaker flask to stirred tank bioreactor
- Great lot to lot reproducibility and reliability