ALiCE® - Simple reaction, radical results. Facile scaling of eukaryotic cell-free protein synthesis from microliters to liter volumes with consistent yields

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INTRODUCTION

Many commercially promising proteins are difficult-to-express (DTE) in living cells and are lost before an industrial production process can be established. Cell-free protein expression (CFPE) is well suited to producing DTE proteins but has largely been limited to microgram yields.

Here we present a eukaryotic lysate, sold as ALiCE® by LenioBio GmbH, which produces protein yields of up to 3mg/ml within a 48 hour reaction. Being a eukaryotic system, ALiCE® has incredible promise for protein engineering due to its capabilities in performing post-translational modifications including disulfide bonds and N-glycosylation.

Recent developments in the scaling of ALiCE® bioprocessing now permit the production of liters of lysate, presenting a new challenge in scaling of the CFPE reaction to match. Herein, we disclose world first liter scale eukaryotic CFPE reactions using commercially available bioreactors.

1. STANDARD ALiCE® WORKFLOW

The ALiCE® cell-free protein expression workflow:

1. Preparation of template DNA by synthesis/cloning of genes in pALiCE plasmids, amplification
2. Thaw lysate, add template DNA, incubate with shaking for 24-48 hours at 25°C.
3. Analyse reaction products and purify protein of interest

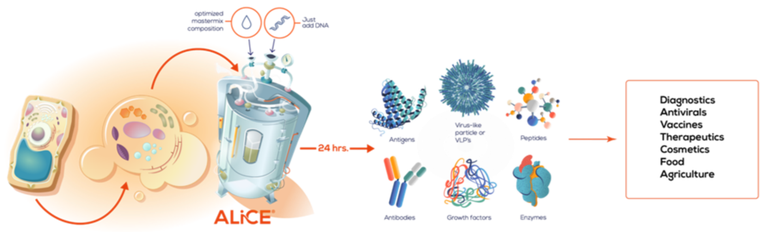


Fig 1. ALiCE is easy to use and capable of producing all classes of protein for use in varied applications

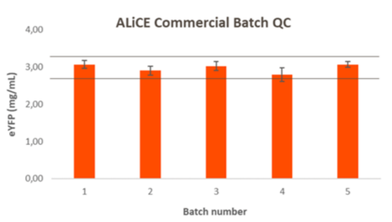
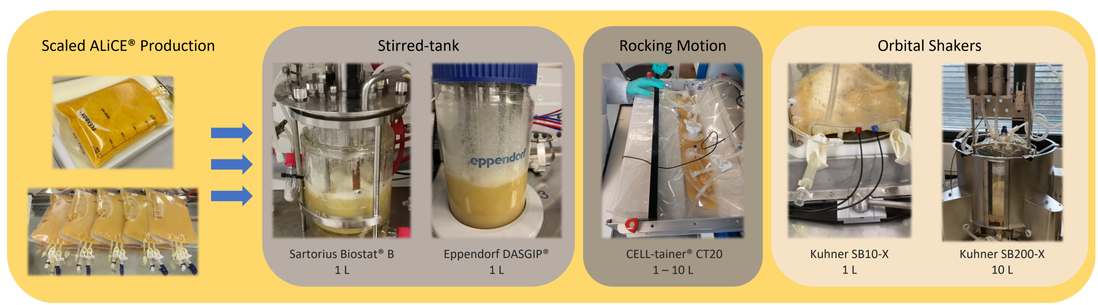


Fig 2. The ALiCE commercial lysate consistently produces eYFP at 3 mg/ml +/- 10%. Shown here, five commercial batches.

2. BIOREACTOR TRIALS

Commercial bioreactors from reputed vendors were trialled for scaled ALiCE® reactions.

Bioreactor conditions were optimized to achieve optimal oxygenation and agitation conditions for high-yielding cell-free protein expression.



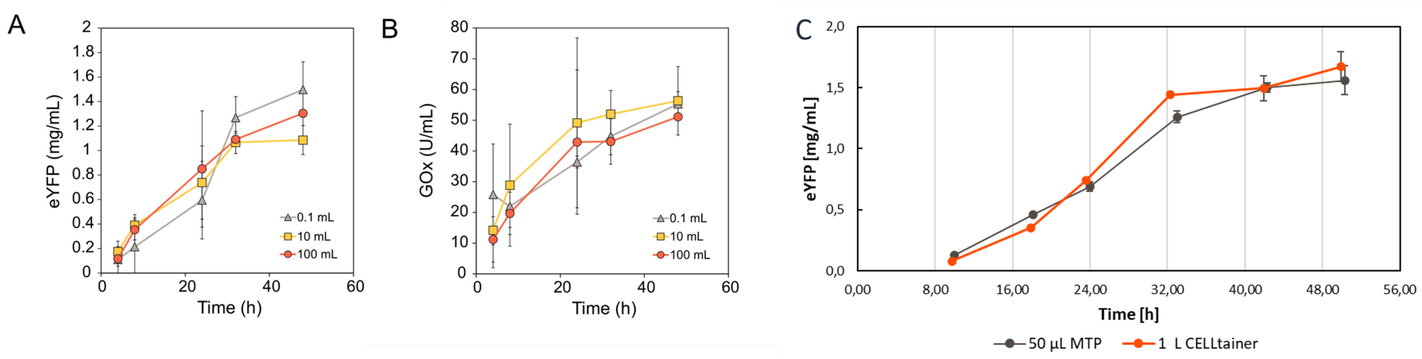
3. LOSSLESS SCALABILITY

Linear scaling of reactions without affecting protein yields:

A. Cytosolic eYFP shake flasks

B. Microsomal glucose oxidase shake flasks

C. 1 L CELLtainer and microtiter plate reactions for eYFP



CONCLUSIONS

1. The ALiCE® lysate is well suited for scaled reactions in a range of off-the-shelf commercial bioreactors

2. Using optimised conditions, lossless scalability across a 20,000x range of reaction volumes was achieved, positioning ALiCE® as an end-to-end protein R&D and production platform

3. Capabilities are in place for scaled production of high-value protein targets for biopharmaceutical, industrial and cosmetics sectors

References
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2. Das Gupta et al. 2022 [Manuscript submitted for publication]
3. www.leniobio.com

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