eppendorf

CASE STUDY No. 004

Cell-Free Expression of a Vaccine Candidate in Scalable Bioreactors

Researchers' affiliations

> **University of Maryland School of Medicine, USA**
> Vaxcyte®, Inc., USA

Equipment

> DASbox® Mini Bioreactor System
> BioFlo® 320 bioreactor control system

Challenge

Shigella spp. are bacterial pathogens, that are a leading cause of moderate to severe diarrhea in young children in low- and middle-income countries. Currently, no licensed vaccine to prevent shigellosis is available.

Invasion plasmid antigens (Ipas) are promising vaccine targets. These components of the ***Shigella* type III secretion system** are highly conserved and therefore vaccines based on them promise to be effective against multiple *Shigella* serotypes.

Scalable production of Ipas is a prerequisite for clinical advancement. The expression and purification of Ipa proteins in conventional cell-based systems have been challenging due to issues with solubility and recovery yields.

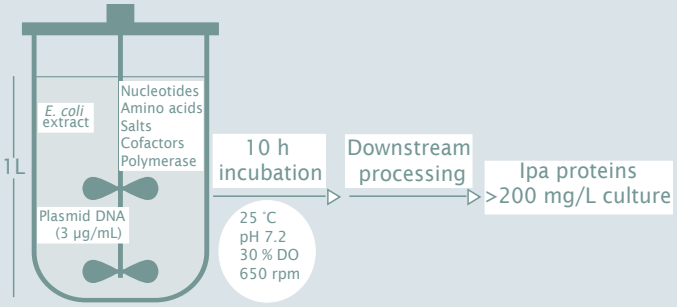
Strategy

IpaB was expressed using **cell-free protein synthesis**. Here, a DNA template is mixed with an *E. coli* extract and components necessary for energy generation and transcription and incubated for 8 to 10 hours.

Process optimization in 96-well format: To optimize IpaB expression, solubility, and recovery yields, various amounts of its chaperone IpgC were co-expressed and added to the reaction mix as purified protein, respectively.

Scale-up in bioreactor: The expression of IpaB was subsequently scaled-up first to a DASbox Mini Bioreactor with a maximum working volume of 250 mL, and then to 1 L using a BioFlo 320 bioprocess controller.

Results



> IpaB was efficiently produced at yields > 200 mg/L.
> The exogenous addition of IpgC to the cell-free expression system enhanced expression, solubility, and recovery yields of IpaB.
> The process was successfully transferred from a 96-well format to a 1-L bioreactor scale.
> Using the same technology, another Ipa protein, IpaH-CTD, was efficiently produced at a 1-L scale.

Conclusion

> Cell-free expression of IpaB led to much higher yields than the ones previously reported for conventional cell-based expression systems.
> The cell-free expression system combined with bioreactors comprised a scalable platform technology for large-scale production of IpaB .
> The technology can be implemented for the expression of other Ipa proteins.

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

Read the full story

Kapoor et al. Efficient production of immunologically active Shigella invasion plasmid antigens IpaB and IpaH using a cell-free expression system. *Appl Microbiol Biotechnol*, 2022