

ESETEC®: HIGH-YIELD PRODUCTION OF AN ACTIVE ANTIBODY FRAGMENT

ESETEC® is an efficient technology for the expression and secretion of an antibody fragment (Fab) into the fermentation broth. The yields of the completely functional and biologically active Fab exceeded 2.2 g/l.

In a joint feasibility study with MorphoSys AG, we assessed that WACKER's ESETEC® secretion expression system is suitable for the microbial production of a Fab that derived from MorphoSys' proprietary Human Combinatorial Antibody Library, HuCAL®. Fabs have similar therapeutical potential to full-length monoclonal antibodies (Mabs). Unlike Mabs, Fabs have successfully been produced by microbial expression, which requires less development and fermentation time, and thus reduces the costs of goods. Fabs from the HuCAL® consist of two different polypeptide chains (light chain, LC, and heavy chain, HC) both of which have intramolecular disulfide bridges (Fig. 1).

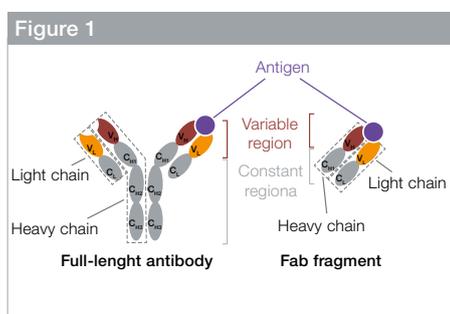


Figure 1: Model of a full-length antibody and an antibody fragment (Fab)

Successful Production of a Fab by Using ESETEC® Features:

- Correct transfer of LC and HC across the inner membrane of *E. coli* into periplasm
- Correct processing, folding and non-covalent assembly of LC and HC into hetero-dimeric Fab molecules in the periplasm
- Fab secretion from periplasm into fermentation broth
- Fab stability in fermentation broth during fermentation
- Advantage of easy and efficient isolation of Fab from fermentation broth
- Full functionality and activity of the extracellularly secreted Fab when compared to the reference Fab produced by secretion into the periplasm
- ESETEC® yields were 40 fold higher than by secretion into the periplasm

How Does ESETEC® Work?

ESETEC® has been designed to secrete recombinant products into the culture broth during fermentation in order to enable very high product yields. The system works by a two-step mechanism:

- In the first step, the target product is transported across the cytoplasmic membrane into the periplasm via the Secpathway. While crossing this membrane, the signal peptide is cleaved off, which releases the native product.
- In the second step, the correctly folded product is uniquely secreted from the periplasm into the fermentation broth across the outer membrane.

The ESETEC® strain derived from *E. coli* K 12 is stable during fermentations in volumes of up to 4,500 l. By a simple cell-separation step, the soluble, native, and active target product can easily be isolated from the cells. Typically yield-decreasing and time-consuming steps of up stream processes such as homogenizing *E. coli* cells, harvesting, solubilizing, and refolding inclusion bodies are unnecessary. When using ESETEC®, the fermentation broth contained the product in concentrations of up to 11 g/l in high initial purity.

Optimizing Yields of Fab

Wacker Biotech uses proprietary expression plasmids to produce the Fab. This series of plasmids encodes the required proprietary signal sequence, and various promoters and origins of replication, too. Optimizing protein yields includes optimizing strain variants and expression plasmids, induction strategies, media components, and other fermentation conditions in shake flasks and fermenters as well. Average yields of functional Fab were > 2.2 g/l in 10 l fed-batch fermentations (see Figures 2 and 3). Yields can be improved even further, by using the high-cell-density fermentation technology, DENSETEC®.

Additional Information

Please also refer to the data sheet: "DENSETEC®, WACKER's High-Cell-Density Fermentation – Optimal Space-Time Yields Combined with High Reproducibility" and to the info sheet "ESETEC®: Unique Protein Production using *E. coli*".

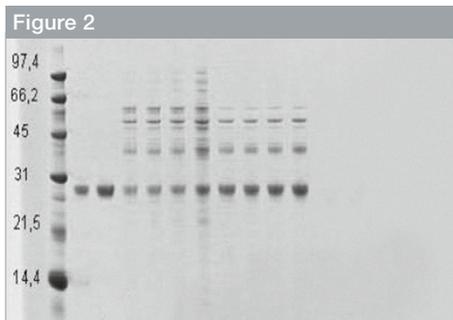


Figure 2: SDS-PAGE of the supernatant of Fab expression tests by using secretion technology strains A and B, resp., protein standard Mw marker; lanes 1 and 2, reference fab (0.5 and 1.0 µg, resp.); lanes 3-6, strain A: first generation secretion strain; lanes 7-10, strain B: second generation secretion strain. Samples were analyzed at four different time points after induction.

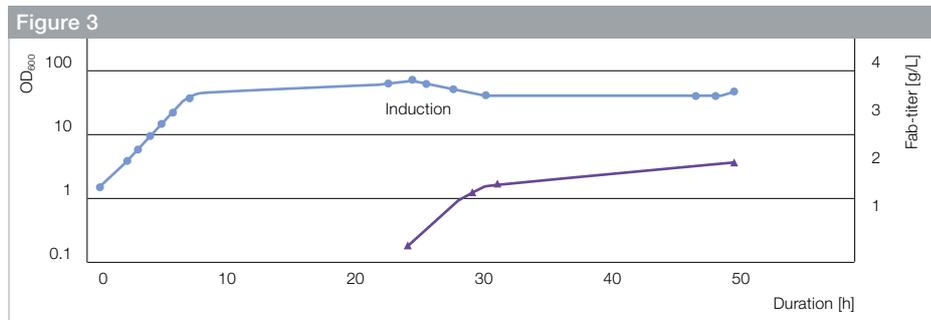


Figure 3: Stable growth of the ESETEC® culture (OD600) and formation of the Fab product over time in a 10 l fermenter (fed-batch fermentation). Circles represent growth, and triangles represent Fab-product formation.

ESETEC® Produces Active Human Fab

The Fab from HuCAL® was easily isolated from the fermentation broth and analyzed. Western Blot showed that LC and HC were expressed at about the same ratio (see Fig. 4). Further analyses by iso-electric focusing, size exclusion chromatography, mass spectrometry, surface plasmon resonance (binding kinetics, Fig. 5), functional cellular assays, and thermal stability (heat, freeze/thaw cycles) showed that the extracellularly secreted Fab and the reference Fab that was produced in the periplasm did not differ in quality. The analytical results confirmed that the Fab folded and assembled correctly, that it was active, and therefore proved that ESETEC® is suitable for producing Fabs in high yield and quality.

Acknowledgement

We would like to thank Dr. R. Ostendorp and his team at MorphoSys AG who contributed to this work.

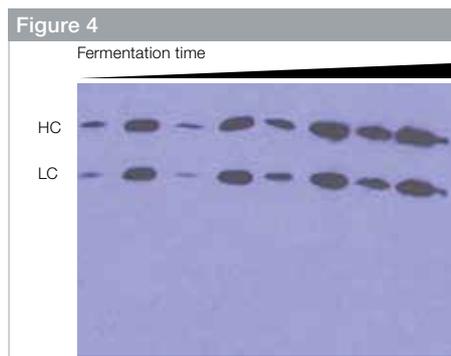


Figure 4: Western Blot showed that LC and HC were expressed at a ratio of about 1:1. The lanes show two different amounts (1 µl and 2 µl, respectively, of a 1:100 dilution) of each sample from the culture supernatant during fermentations at four different time points after induction. Blotted gels were run under non-reducing conditions that allowed LC and HC to be separated.

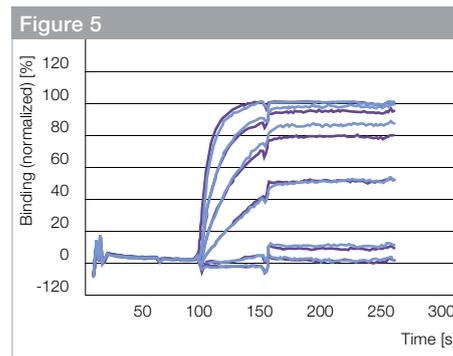


Figure 5: The quality of the secreted Fab equals the benchmark Fab. The Fab that was produced by ESETEC® (purple graphs) bound to an immobilized antigen target with the same binding kinetics as the benchmark Fab from MorphoSys AG that was produced in the periplasm (blue graphs) as analysis by surface plasmon resonance (BIAcore) proved. This figure was kindly provided by MorphoSys.

Availability

WACKER Biotech's contract manufacturing service for clients uses cGMP-compliant ESETEC® technology. MorphoSys offers partners antibody generation programs based on ESETEC® technology in order to produce antibodies on a research scale at MorphoSys. Please contact us for more information.

