CASE STUDY
PRODUCTION OF A HuCAL® FAB FRAGMENT USING THE WACKER SECRETION SYSTEM

The WACKER Secretion System has proved highly effective in the expression of a Fab fragment. Yields of secreted functional Fab fragment exceeded 2g/L in the fermentation broth.

In a feasibility study, MorphoSys, a leading biotechnology company in the area of fully human antibodies, commissioned WACKER Biotech with the production of a HuCAL® antibody drug candidate (Fab fragment) with WACKER's E. coli-based Secretion System.
Fab fragments combine advantages of antibodies in therapy with the possibility of microbial-based production, thus reducing the production costs and fermentation times.
HuCAL® Fab fragments are assembled of two different sub-units and have intra-molecular disulfide bridges.

For the production of the Fab fragment with the WACKER Secretion System:
- Two different polypeptide chains are secreted across the inner membrane of E. coli.
- The two chains are processed correctly and assemble non-covalently.
- The Fab fragment is transferred into the culture broth.
- The Fab fragment is stable in the medium from where it can be purified efficiently.
- The extra-cellularly secreted Fab fragment shows full functionality as compared with its counterpart produced by secretion into the periplasm.

The Principle of the WACKER Secretion System

The WACKER Secretion System has been designed to transfer recombinant proteins during fermentation in very high yields into the culture medium. The system is based on a two-step export mechanism:

- First, the sec-pathway is used to transport the target product across the cytoplasmic membrane into the periplasmic space. During this step the signal peptide is cleaved off, releasing the native product.
- The second step is mediated by a unique feature of the proprietary WACKER Secretion Strain. The correctly folded product is translocated from the periplasmic space across the outer membrane into the culture broth. The strain is an E. coli K 12 derivative and proved to be stable in large scale fermentation of 4500 L.

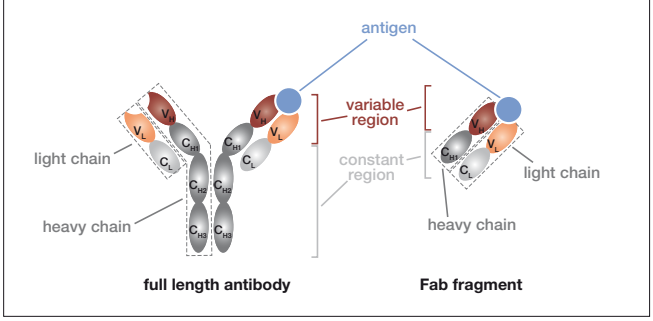
By means of a simple cell-separation step, the target product can be purified from the culture broth in a soluble, native and active form. Typical yield-decreasing and time-consuming steps of E. coli processes, such as cell homogenization, inclusion body harvesting, solubilization and refolding, are not necessary. Product yields as high as 7g/L in the culture medium have already been obtained with the Secretion System, and the initial purity is high.
Please also refer to the data sheet: The WACKER Secretion System- an E. coli Protein production system with unique properties.

Optimization of the Production System

WACKER Biotech uses its proprietary expression plasmids to produce the Fab fragment. The plasmid comprised various elements from a toolbox especially tailored to the Secretion System, for example a very effective proprietary signal sequence.

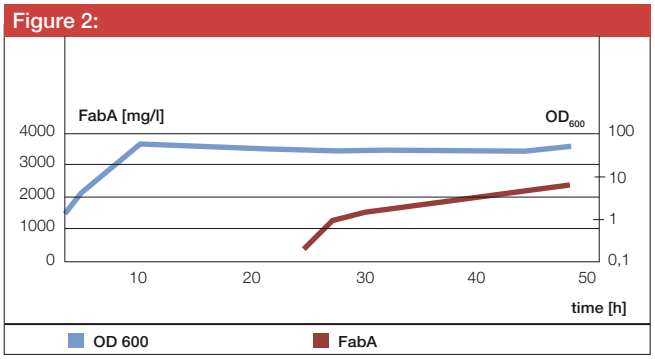
Optimization work included the expression plasmid, induction strategies, media components and other cultivation conditions in shake flasks and fermenters. Average yields of functional Fab fragment of more than 2g/L have been reached in 10 L fed-batch fermentations. (see Fig. 2)

Figure 1:



Schematic of a full length antibody and a Fab antibody fragment.

Figure 2:



Growth curve and production of the HuCAL® Fab fragment by the WACKER Secretion System in a 10 L fed-batch fermentation run.

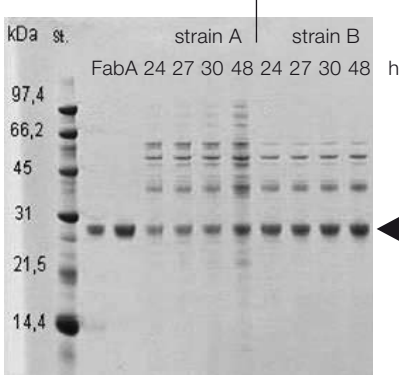
Functionality of the HuCAL® Fab Fragment

The Fab fragments were easily purified from the culture medium. Western Blot data indicate a balanced expression of heavy and light chain (see Fig. 4).
Further intensive analyses have been performed at MorphoSys and WACKER. Comparisons with the reference Fab fragment from periplasmic expression show no detectable differences as measured e.g. by:

- Iso-electric focusing,
- Size exclusion chromatography,
- Mass spectrometry,
- Binding kinetics,
- Functional cellular assays,
- Stability upon thermal stress (heat, freeze/thaw cycles)

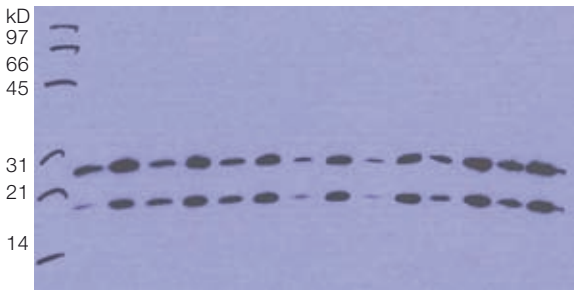
All data indicate correct folding and assembly of the Fab fragment and confirm the suitability of the WACKER Secretion System for producing Fab fragments at high yield in the culture broth.

Figure 3:



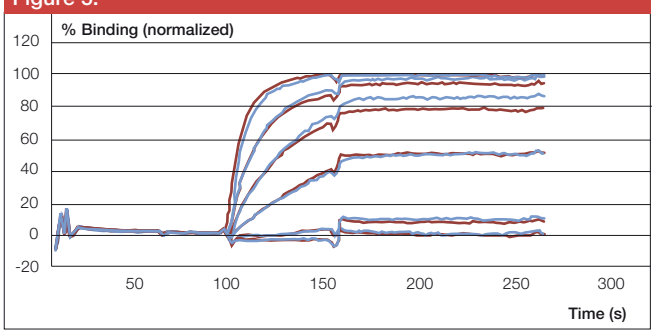
Production of the Fab fragment in two different WACKER Secretion Strains over time. In each case, 0.2 micro liter of culture supernatant taken during fermentation at different points in time is applied to a 12% reducing SDS-PAGE. The arrow highlights the two produced Fab chains.

Figure 4:



Balanced expression of heavy and light chain of the Fab fragment. Culture supernatants were analysed by non-reducing SDS-PAGE and Western Blot, using detection antibodies against light and heavy chains.

Figure 5:



Analysis of binding kinetics of the HuCAL® Fab fragment to immobilized antigen using surface plasmon resonance (BIAcore). (red: WACKER material, blue: reference material)

Availability

WACKER Biotech makes this Secretion System available as a service to its clients for contract manufacturing of the client's products according to cGMP.
MorphoSys makes this Secretion System available as a service to its partners within antibody generation programs at MorphoSys for production of antibodies at research scale.

Contact

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