THE WACKER SECRETION SYSTEM – AN E. COLI PROTEIN PRODUCTION SYSTEM WITH UNIQUE PROPERTIES

Wacker Biotech provides its clients with an innovative and highly efficient E. coli expression system that results in the secretion of the recombinant product in native conformation into the culture broth. The system therefore simplifies the primary recovery and purification processes. So, right from the start, our clients are able to choose a cost-efficient system for manufacturing their products.

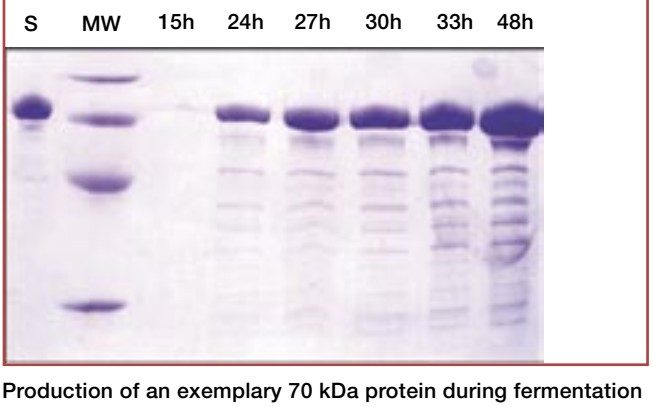
The Principle

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

- First, the target product is transported across the cytoplasmatic membrane into the periplasmatic space via the sec-pathway. 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 secreted from the periplasmatic space across the outer membrane into the culture broth.

By way of a simple cell-separation step, the target product can therefore be isolated in a soluble, native and active form from the culture broth. It is present in high purity, and yields as high as 7 g/l have already been obtained (see Fig. 1 and 3).

Figure 1:



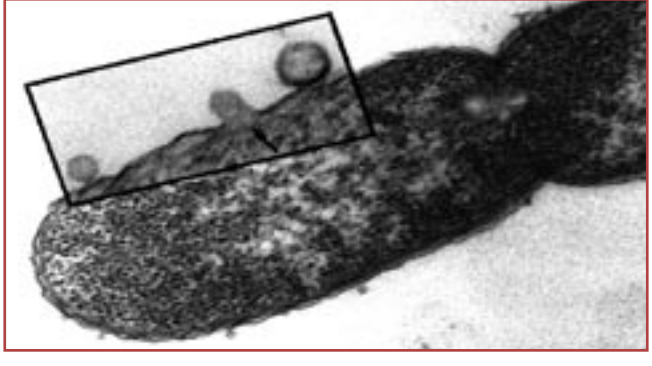
Production of an exemplary 70 kDa protein during fermentation over time. 2 microliters of culture supernatant taken during fermentation at different points in time are applied to an SDS-PAGE.

The WACKER Secretion Strain

The most important component of the WACKER Secretion System is an especially optimized production strain with the following properties:

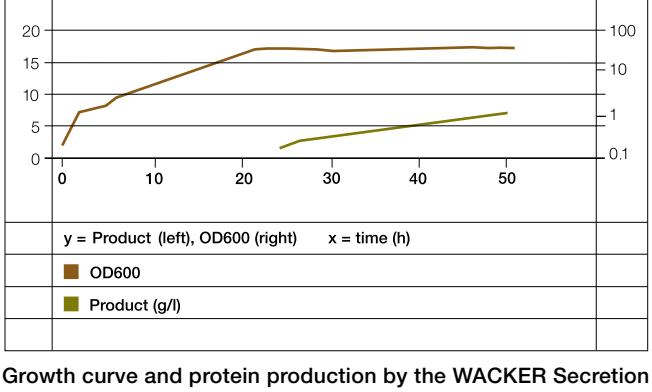
- E. coli K12 derivative, biosafety level 1
- genetically well characterized
- optimized for secretion by a modified outer membrane (see Fig. 2)
- stable in commercial-scale fermentation (see Fig. 3)
- easy to handle for molecular biology operations
- different genetic variants available, e.g. protease deletion mutants
- intellectual property of WACKER

Figure 2:



Electron micrograph of a single cell of the WACKER Secretion Strain

Figure 3:



Growth curve and protein production by the WACKER Secretion Strain on a 4.5 m^3 fermentation scale.

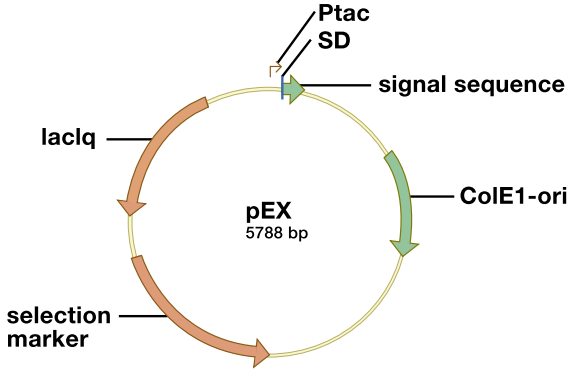
The Expression Plasmids

There is a toolbox of various expression plasmids for the WACKER Secretion System which show the following features (see Fig. 4):

- different origin of replications resulting in different copy numbers for fine-tuning the expression level
- tac promoter system including also the laclq repressor
- different signal sequences: very effective WACKER proprietary signal sequences and standard signal sequences such as phoA, ompA, pelB

Alternatively, plasmids designed by our clients can be used.

Figure 4:



Plasmid map of a typical expression plasmid of the WACKER Secretion System.

Additional Elements of the WACKER Secretion System

Several helper elements are available to optimize expression, solubility or secretion of the target recombinant product. These elements may be introduced into the system either as a second gene on the expression plasmid or encoded on helper plasmids added along with the expression plasmid. The helper plasmids use a kanamycine resistance gene for selection.

Helper elements include:

- cytoplasmatic chaperones
- components of the secretion apparatus
- periplasmatic chaperones
- disulfide bridge formation factors

The Target Molecule

The WACKER Secretion System is suited for the production of recombinant products with a wide range of properties:

- prokaryotic or eukaryotic origin
- wide range of molecular weight and pI
- fusion proteins or native proteins
- proteins with amino acids that differ from methionine at position 1
- proteins with disulfide bridges

Up to now, the WACKER Secretion System has been used successfully for the production of various enzymes and proteins, antibody fragments and peptides.

The Expert's Opinion

The system has been evaluated by a well-known industry expert. Please ask for the expert report evaluating the WACKER Secretion System.

Availability

Wacker Biotech makes this Secretion System available as a service to our clients for manufacturing the client's product according to cGMP. We bring years of experience with the system to your project.

Please contact Wacker Biotech for more information:

Dr. Anke Suter
Business Development & Sales Manager
Phone: +49 3641 579-920
Fax: +49 3641 579-921

Wacker Biotech GmbH
Hans-Knöll-Straße 3
07745 Jena, Germany
info.biologics@wacker.com
www.wacker.com/biologics

6198/06.05e/1500