

ESETEC®: UNIQUE PROTEIN PRODUCTION USING *E. COLI*

Wacker Biotech's ESETEC® technology provides clients with an innovative and highly efficient *E. coli* expression system, which enables secretion of native recombinant protein products into the fermentation broth. This simplifies primary recovery and purification processes. So, right from the start, our clients have a cost-efficient system for manufacturing their products at their disposal.

The ESETEC® Mechanism

ESETEC® has been designed to secrete recombinant products into the culture broth during fermentation in order to enable very high yields.

The system is based on a two-step mechanism:

- In the first step, the target product is transported across the cytoplasmic membrane into the periplasm via the Sec-pathway. While crossing the membrane, the signal peptide is cleaved off, which releases the native product.
- In the second step, the correctly folded product is secreted from the periplasm into the culture broth across the outer membrane. This secretion is a unique feature of the proprietary ESETEC® Secretion Strain.

By a simple cell-separation step, the soluble, native, and active target product can easily be isolated from the fermentation broth. At this stage, the product is already highly pure and yields are up to 11 g/l (see Fig. 1 and 2).

The ESETEC® Secretion Strain

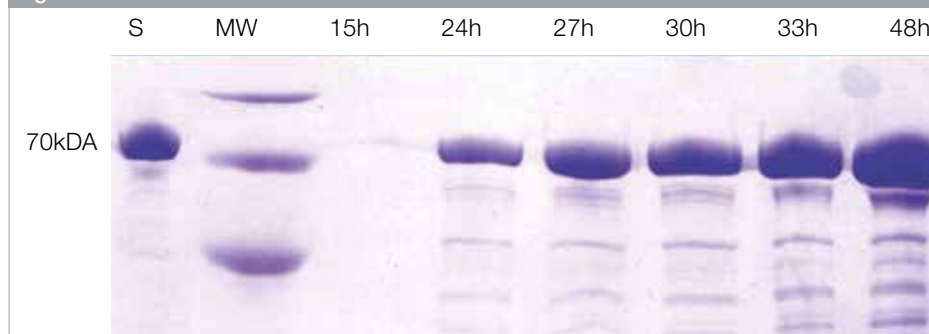
ESETEC®'s most important component is a specifically optimized production strain with the following properties:

- *E. coli* K12 derivative, biosafety level 1
- Genetically well characterized
- Optimized for secretion by a modified outer membrane
- Easy to manage in molecular biology operations
- Available in different genetic variants, e.g. with protease deletion mutants (see Fig. 2)
- WACKER's intellectual property

Electron Micrograph of Single Cells of the ESETEC® Secretion Strain

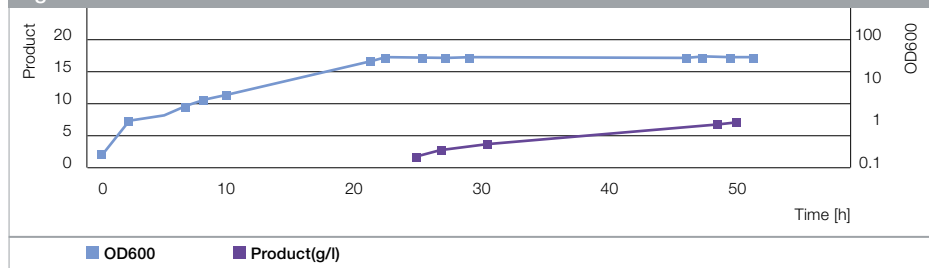


Figure 1



Production of an exemplary protein over fermentation time. Two microliters of culture supernatant were sampled during fermentation at different time points and then analyzed by SDS-PAGE.

Figure 2



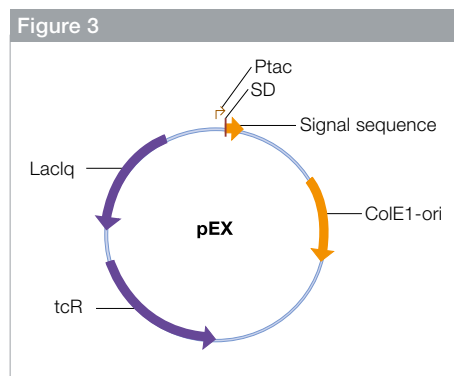
Graphs of bacterial cell growth and protein production as a function of fermentation time using the ESETEC® Secretion Strain on a 4.5 m³ fermentation scale.

The Expression Plasmids

In order to customize protein production, a toolbox has been created which includes various expression plasmids for the ESETEC® system (see Fig. 3).

These plasmids feature:

- Different origins of replication that result in different copy numbers for fine-tuning the expression level
 - The tac promoter system including also the laclq repressor
 - Different signal sequences: These are very effective WACKER proprietary signal sequences or standard signal sequences such as phoA, ompA, pelB
 - The tetracycline resistance gene
- Alternatively, plasmids designed by clients can be used.



Plasmid map of a typical expression plasmid of the ESETEC® Secretion System

Additional Helper Elements of the ESETEC® 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 another gene on the expression plasmid or as a gene on a helper plasmid that is transformed along with the expression plasmid.

Helper elements include:

- Cytoplasmic chaperones
- Components of the secretion apparatus
- Periplasmic chaperones
- Disulfide bridge formation factors

Various Target Molecules

ESETEC® is suitable for the production of:

- Proteins of prokaryotic or eukaryotic origin
 - Proteins with a wide range of molecular weight and pl
 - Fusion or native proteins
 - Proteins with amino acids that differ from methionine at position 1
 - Proteins with disulfide bridges
- Up to now, ESETEC® has successfully produced various peptides, enzymes, and proteins such as antibody fragments and scaffolds. ESETEC® derived products have been approved for clinical trials.

Additional Information

Please also refer to the info sheets “Case Study – ESETEC®: High-Yield Production of an Active Antibody Fragment” and “Case Study Protein Scaffolds – ESETEC®: Efficient Manufacturing of a Pegylated Anticalin®”.

The Expert's Opinion

ESETEC® has been evaluated by a well-known industry expert. Please ask for the expert evaluation report on ESETEC®.

Availability

Wacker Biotech provides ESETEC® technology for cGMP-compliant manufacture of its clients' products. Your projects will profit from our years of experience with the system.

Please contact us for more information.



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