WACKER

CREATING TOMORROW'S SOLUTIONS

WACKER BIOTECH I SECRETION TECHNOLOGY I ESETEC®

NEXT GENERATION ESETEC®: CONTROLLED SECRETION OF BIOPHARMACEUTICALS

With ESETEC®, Wacker Biotech provides an innovative and highly efficient *E. coli* expression system, which enables controlled secretion of correctly folded recombinant protein products into the fermentation broth. This simplifies primary recovery and purification processes. ESETEC® is a best-in-class manufacturing platform for non-glycosylated biopharmaceuticals.

The ESETEC® Secretion Strain

The key component of ESETEC® is a proprietary, specifically optimized production *E. coli* K12 strain designed to secrete the desired product across both *E. coli* membranes into the culture broth. The technology is easy to integrate and control in biotech operations and is stable in commercial-scale fermentation. ESETEC® has been approved by EMA and FDA for clinical supply. WACKER continuously improves ESETEC® technology to meet the specific needs of each protein. In addition to different genetic variants, e.g. with protease deletion mutants and antibiotic-free selection, WACKER now offers the newest ESETEC® strain with **fully controlled product secretion** into the culture broth. The strategy of controlled secretion makes ESETEC® especially suitable for **difficult-to-produce proteins**, where other expression systems were not successful.

Various Target Molecules

ESETEC® is suitable for the manufacture of difficult-to-produce:

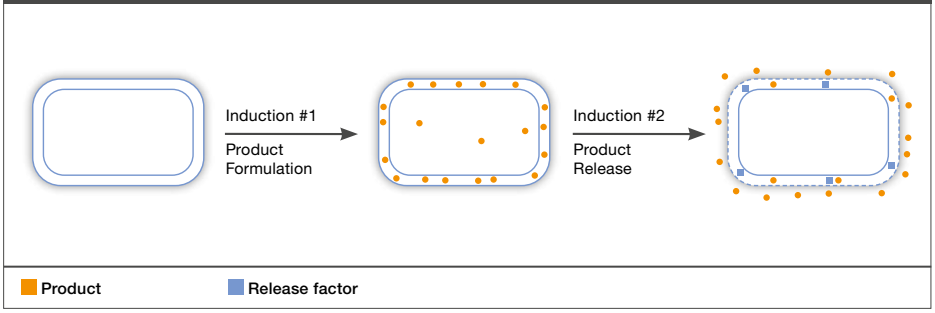
- Proteins of prokaryotic, eukaryotic or artificial origin
- Proteins with a wide range of molecular weights (5 kD – 150 kD) and isoelectric points
- Monomers – tetramers – heterodimers
- Fusion or native proteins
- Proteins with authentic N-termini and different starting amino acids
- Proteins with multiple disulfide bridges
- Novel antibody formats (e.g. single-domain antibodies) and antibody fragments (e.g. Fab)
- Scaffolds
- Peptides
- Enzymes
- Growth factors (e.g. hGH)

Advantages of ESETEC® over Mammalian Cells (CHO) for Non-Glycosylated Proteins

ESETEC® and CHO both secrete correctly folded target protein into the fermentation broth. However, as a microbial system, ESETEC® is unrivaled with regard to costs:

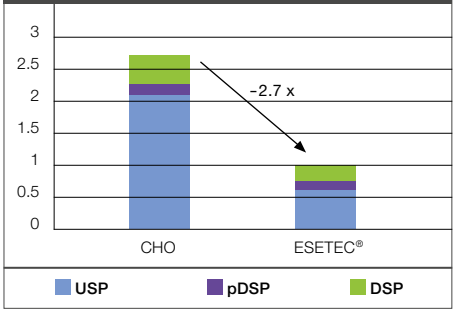
- Process development with ESETEC® is faster due to straightforward strain development
- No development, validation or analytics for virus inactivation and filtration needed
- The fermentation time is up to 10-times shorter than with CHO systems, resulting in higher daily productivity and reduced batch duration
- Up to 2-times lower costs for the manufacture and release of microbial GMP cell banks
- Up to 3-times lower cost of goods based on process simulations (assuming identical titers and process yields)

Process of Controlled Microbial Secretion



The principle: after a production phase and accumulation of the product in periplasm, the addition of an inductor actively starts the release of the product into the culture broth

Costs Relative to ESETEC®



Total costs of single GMP batches relative to ESETEC®. The cost of goods is reduced up to 2.7-fold due to shorter process times, reduced media costs, and viral depletion steps.

WACKER

CREATING TOMORROW'S SOLUTIONS

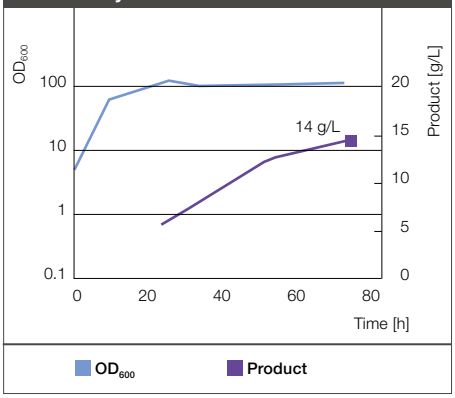
ESETEC® Simplifies Purification: Highest Titers of Purest Product

In a single cell-separation step, the soluble and biologically active target protein can easily be isolated from the fermentation broth. At this stage, the product is already highly pure, and yields are up to 14 g/L.

Case Studies with Difficult-to-Produce Proteins: How to Crack a Hard Nut

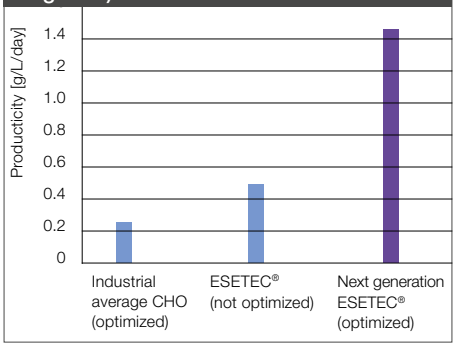
The following graphs demonstrate the ability of the newest ESETEC® strain to produce even very difficult-to-manufacture complex therapeutic proteins such as new antibody formats with high titers. The findings also show that the new ESETEC® version – even without full process development – offers advances in productivity that exceed industry-optimized processes with mammalian cell cultures (CHO cells). Another advantage of the ESETEC® secretion approach is reduction of host impurities – up to 1,000-times lower load of host cell proteins/DNA compared to classical *E. coli* strains.

Case Study "Hirudin"

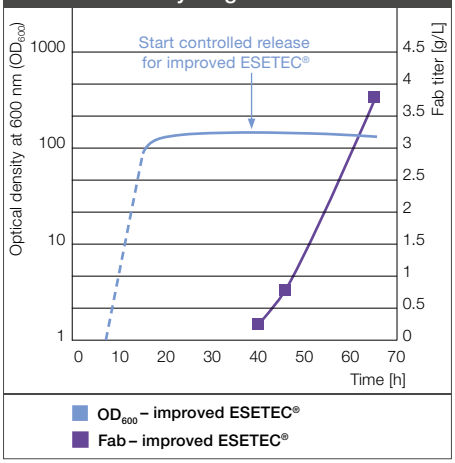


Hirudin is a complex therapeutic coagulant derived from leech with three disulfide bridges. ESETEC® secretes 14 g/L hirudin with full functional activity.

Upstream Productivity for a Non-Platform Protein (Difficult-to-Produce Antibody Fragment)



Secretory Production for a Difficult-to-Produce Antibody Fragment with ESETEC®



Test Your Protein with the ESETEC® System

We offer feasibility studies to test the suitability of the ESETEC® system for the expression and secretion of soluble, correctly folded target protein into the culture supernatant. The study includes screening of different ESETEC® hosts, plasmids and helper proteins to find the best performing strain candidate. Additionally, various cultivation conditions are tested in parallel in multiple bioreactors (3 – 5 L scale). The customer receives analytical results and on request, a cell-free product containing supernatant (optionally purified using a generic chromatography step).

ESETEC®

Wacker Biotech GmbH, Jena and Halle (Germany), Phone: +49 3641 5348-0, **Wacker Biotech B.V.,** Amsterdam (the Netherlands)
Phone: +31 20 750 3600, info.biologics@wacker.com, www.wacker.com/biologics
Follow us on:

7405e/02.21 replaces 7405e/09.16