

# ESETEC® – UNITING BENEFITS OF CHO AND MICROBIAL SYSTEMS

With ESETEC®, Wacker Biotech provides an innovative and highly efficient *E. coli* expression system, which enables the 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

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
- Approved by EMA and FDA for clinical supply
- Secretion of desired product across both *E. coli* membranes into the culture broth
- Easy to integrate and control in biotech operations
- Available in different genetic variants, e.g. with protease deletion mutants and antibiotic-free selection
- Stable in commercial-scale fermentation
- Intellectual property of WACKER

## Various Target Molecules

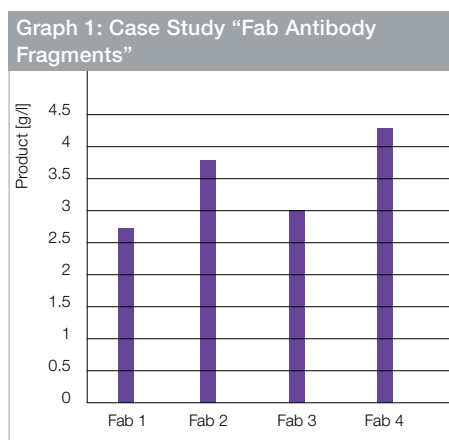
ESETEC® is suitable for the production of:

- Proteins with 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 start 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)

To further improve productivity for state-of-the-art therapeutic antibody fragments, WACKER recently developed and released the next generation of its secretion technology – ESETEC® 2.0. Targeted genetic modifications and process optimization measures have led to the development of new, extremely productive cell lines and fermentation procedures.

Fabs can now be produced in yields of several grams per liter and can be secreted into the culture medium in active form (see Graph 1).



Case study with four different Fabs of high clinical relevance. ESETEC® 2.0 secretes several g/l of fully functional Fabs into the culture broth.

## ESETEC®'s Advantages over Mammalian Cells (CHO) for Non-Glycosylated Proteins

ESETEC® and CHO both secrete correctly folded target protein into the fermentation broth.

Their common benefits are:

- High product quality and titers
- Simple purification due to low content of process-related impurities including endotoxins and host cell proteins

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 with the *E. coli* system than with CHO systems, resulting in higher daily productivity and significantly reduced batch duration
- Up to 3 times lower cost of goods based on process simulations (assuming identical titers and process yields)
- Microbial seed trains require less fermenters, which translates to lower capital costs compared to cell culture facilities
- Up to 2 times lower costs for the manufacture and release of a microbial GMP cell bank

**The advantages of ESETEC® at a glance: Highly cost-efficient production system thanks to superior productivities, shorter development timelines and lower cost of goods.**

For more information about the COGS analysis please see our publication in GEN (May 2017) "Microbial secretion via ESETEC® technology".

**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:

- Step 1: The target product is actively transported across the cytoplasmic membrane into the periplasm.
- Step 2: 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 WACKER secretion strain.

In a simple 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.

**Additional Helper Elements of the ESETEC® System**

Several helper elements are available to optimize expression, solubility, secretion or folding of the target product. These helper elements include:

- Cytoplasmic chaperones
- Components of the secretion apparatus
- Periplasmic chaperones
- Factors promoting proper protein folding

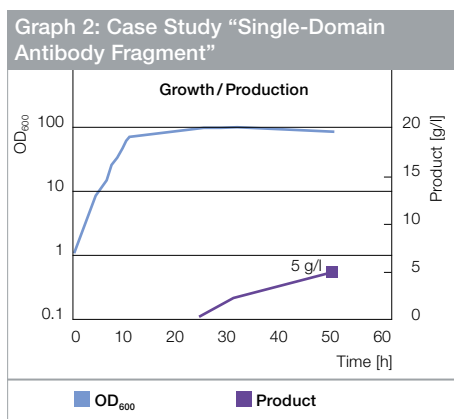
**The Expression Plasmids**

There are various expression plasmids available for ESETEC®:

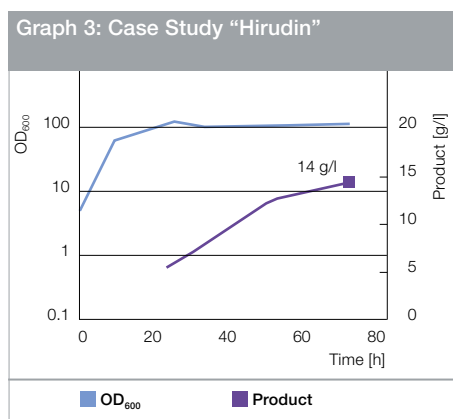
- Different origins of replication for fine-tuning the expression level
- The tac promoter system including the lacIq repressor
- Different signal sequences: very effective proprietary signal sequences or standard signal sequences such as phoA and ompA
- Antibiotic-free selection and manufacture (based on transfer of essential genes)

**Case Studies with ESETEC® Expression System**

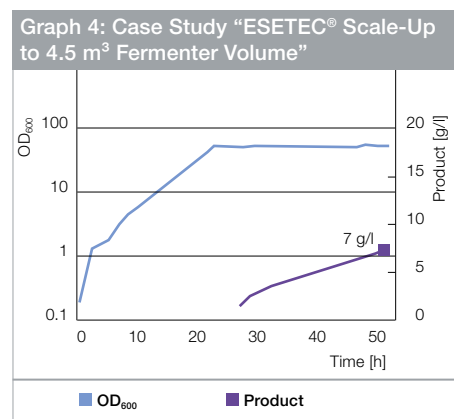
The following three graphs demonstrate the scalability of the production process with ESETEC® and its ability to produce high titers of complex therapeutic proteins such as new antibody formats.



ESETEC® secretes the single-domain antibody (sdAb) with yields of 5 g/l on a 1-l fermentation scale.



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



Scale-up of the ESETEC® process for production of an enzyme on a 4,500-l fermentation scale – good growth and over 7 g/l of secreted product.

