WACKER

CREATING TOMORROW'S SOLUTIONS

WACKER BIOTECH I REFOLDING TECHNOLOGY I FOLDTEC®

FOLDTEC®: HIGHLY EFFICIENT PROTEIN REFOLDING AND UNIQUE *E. COLI* EXPRESSION SYSTEM

Refolding is the art of transferring denatured and misfolded recombinant proteins back into their biologically active form. This is of particular importance for complex biopharmaceuticals which cannot be produced in their soluble state (e.g. small, hydrophobic, toxic or highly complex proteins). Wherever *in vitro* refolding is needed for recombinant manufacturing of such challenging pharmaceutical proteins, WACKER offers its novel FOLDTEC® technology. It comprises highly efficient *E. coli* producer strains, an antibiotic- and phage-free plasmid maintenance system and comprehensive refolding know-how.

The expression of recombinant thrombin as inclusion bodies and elaboration of an optimized *in vitro* refolding regime are demonstrated in a case study on the back page. Thanks to WACKER's expertise, a 5-fold increase in yield and 4-times reduction of working volume were achieved, corresponding to 20-fold higher productivity.

Refolding as a Key Production Stage

While WACKER's own ESETEC® technology has proven highly efficient in producing soluble proteins and antibody fragments via secretion, poorly soluble substances form aggregated inclusion bodies within the cell, which contain incorrectly or incompletely folded target molecules. So, protein refolding is a key production stage in order to achieve the desired active properties.

The Challenges of Refolding

Various physical parameters, additives and buffer components influence the outcome and efficacy of refolding processes. Selection and proper adjustment of these conditions is of utmost importance for the cost-efficient industrial manufacturing of biopharmaceuticals.

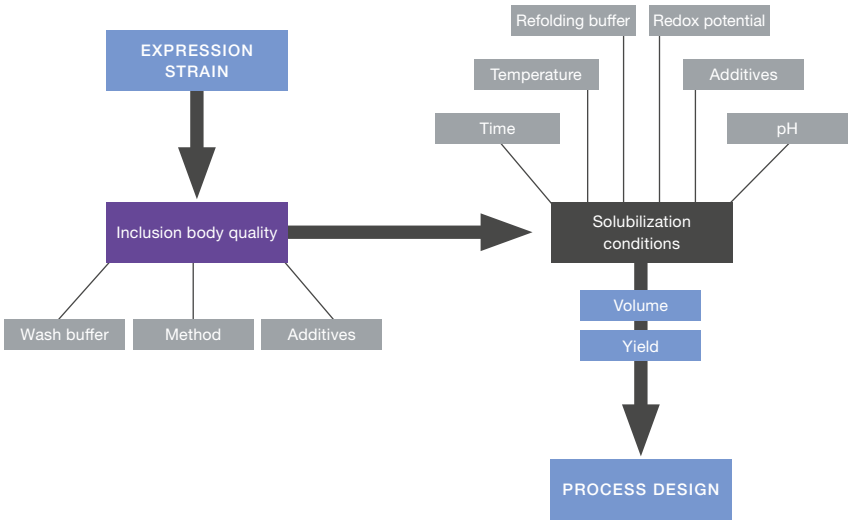
Many refolding protocols show poor efficacy or cannot be transferred to commercial scale – either due to extremely high working volumes or expensive buffer additives. Screening to identify the most suitable combinations can be tedious and time consuming. It requires an in-depth understanding of protein folding and the technical requirements and limitations of large-scale manufacturing.

The Solution: FOLDTEC®

With all its experience, WACKER has developed a technology to meet refolding's challenges. Based on optimized and proprietary *E. coli* strains in combination with a patented plasmid maintenance system and years of experience in designing refolding processes, WACKER can now cost-efficiently and reliably produce aggregating pharmaceutical proteins in high yields and purities.

In combination with a set of plasmids, the system shows high expression levels without the need for antibiotics or phage components. Supported by WACKER's patented high-cell-density processes, expression results in high-quality inclusion bodies containing up to 12 grams of target protein per liter culture broth.

Identification of Optimum Refolding Conditions in a Multi-Parameter Space



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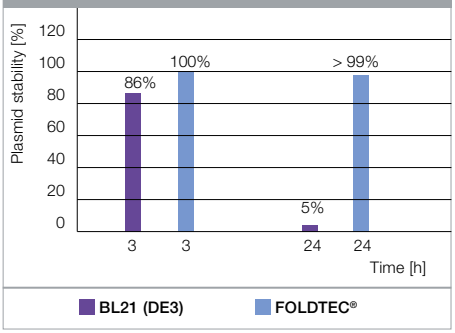
Antibiotic- and Phage-Free

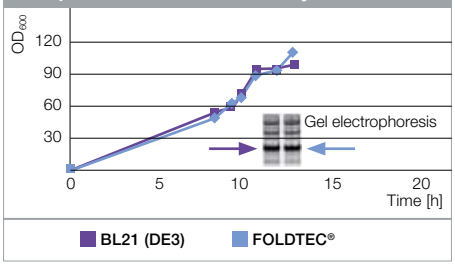
While conventional systems usually depend on antibiotics, FOLDTEC® can do without them entirely (see Graphs 1 and 2). It also completely avoids phage components, which are generally needed by comparable systems. Both of these aspects contribute to the new technology's high safety and environmental compatibility and meet the strictest of regulatory requirements.

Superior Plasmid Stability without the Need for Antibiotics



Graph 1: Comparable *E. coli* systems like BL21 (DE3) rely on antibiotics to ensure genetic stability. FOLDTEC®'s innovative plasmid maintenance system can do without them, which is in line with the strictest of regulatory requirements.

Similar Growth and Gene Expression Compared to Conventional Systems

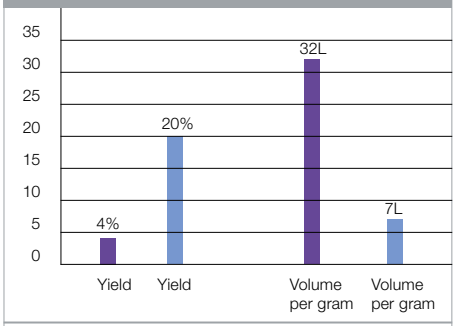


Graph 2: Fed-batch fermentation of the public BL21 (DE3) and the FOLDTEC® systems – similar growth kinetics and gene expression. Higher safety without compromising on yields.

Case Study: Expression of Recombinant Thrombin

To demonstrate the tremendous potential for cost reduction, WACKER applied its refolding strategy to recombinant thrombin. Thrombin is a central enzyme in the coagulation cascade with an important role in medicine and surgery. Transferring this serine protease into its active conformation is challenging; published yields are extremely low and refolding requires large working volumes.

Case Study – Optimizing *In Vitro* Refolding of Recombinant Thrombin



Graph 3: Compared to a state-of-the-art process, folding optimization increased space/time yields 20-fold.

After completion of the folding optimization, WACKER had increased yields 5-fold, while reducing the volume per gram thrombin by more than 4 times (see Graph 3). This corresponds to a 20-fold increase in productivity.

For each individual refolding project, a crucial success factor is the establishment of solid and reliable analytics. By developing a tailor-made chromatography method and activity assay, WACKER has obtained powerful tools to distinguish between functional and misfolded thrombin. Rational experimental design identified a novel chaotrope for a significant improvement of yields. Finally, working volumes were reduced by so-called pulse refolding, which allows higher protein concentrations and renders the process suitable for commercial manufacturing.

Features of the New FOLDTEC® Technology

- Proprietary *E. coli* host system for high-level inclusion body expression (titers up to 12 grams per liter)
- Patented antibiotic- and phage-free plasmid maintenance system
- Free choice of culture media (with or without complex media components)
- Extensive knowledge in refolding – fully aligned with our expression strains
- Up to 20-times increase in productivity (space/time yields) demonstrated for refolding of recombinant thrombin
- Patented high-cell-density processes ideally suited for inclusion body manufacturing

FOLDTEC®

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