

One-Stop-Shop Protein Recovery from Inclusion Bodies

Speed your time to market

The increasing demand for biotherapeutics as well as healthcare cost pressures have spurred an intense search for more cost effective, faster and more efficient alternative expression systems.

Whereas CHO cells continue to be the industry workhorse for mammalian manufacturing, microbial expression systems such as yeast and bacteria have gained a significant market share in manufacturing simpler molecules such as peptides, antibody fragments and derivatives thereof.

Expression in *E. coli* of less complex molecules can be achieved faster and at a reduced cost, with a significantly higher upstream productivity than in mammalian biomanufacturing.

Our portfolio consolidates dual-sourced Emprove® chemicals/enzymes to facilitate our customers' regulated microbial manufacturing processes. CellPrime® process enzymes complement the offering with unrivaled transparency of their recombinant, non-animal origin (NAO) production process. Our *E. coli* expression portfolio is geared towards ascertaining, managing and mitigating risk on your journey to qualify,

expedite and advance your manufacturing process towards regulatory approval. Expect to be furnished a toolbox for mastering each challenge encountered when *E. coli* expressed foreign proteins need to be extracted and refolded from inclusion bodies.

The utility of these components can easily be demonstrated by mapping them to individual steps in the manufacturing workflow for therapeutic recombinant insulin (shown below)

The conventional strategy for this manufacturing process encompasses the following major steps:

- Induction of heterologous expression **(A)**
- Cell harvest and lysis **(B)**
- IB purification **(C)**
- IB protein extraction **(D)**
- Protein refolding **(E)**
- r insulin precursor intermediate maturation **(F)**

Production of recombinant insulin in *E. coli* and purification from IBs

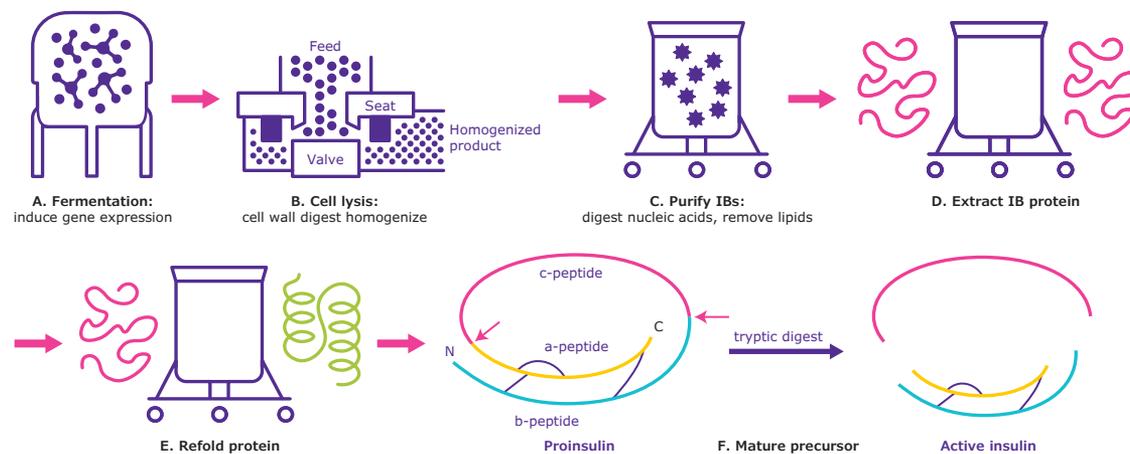


Figure 1. Manufacturing of therapeutic recombinant insulin as a model for *E. coli* IB expressed molecules. The chemicals and recombinant enzymes used are listed in the table below for each step A–F.

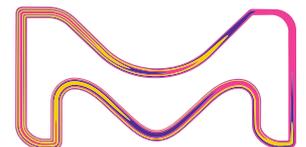


Table 1. Products suitable for inclusion body protein induction, extraction, and refolding

Step	Product #	Description	Function
A	137064	Isopropyl-β-D-1-thiogalactopyranoside Emprove® EXPERT	Induce gene expression
A	PHG0010	Isopropyl-β-D-1-thiogalactopyranoside	Induce gene expression
B	5501	CellPrime® r lysozyme recombinant human lysozyme	Digest murein sacculus
C	101697	Benzonase® endonuclease Emprove® Expert: for nucleic acid removal in biopharmaceutical production	Digest nucleic acids trapped in IBs
C	103773	Benzonase® endonuclease Safety Plus Emprove® Expert: for nucleic acid removal in biopharmaceutical production	Digest nucleic acids trapped in IBs
C	108643	Triton® X-100 Emprove® EXPERT Ph.Eur.	Solubilize lipids trapped in IBs
D	PHG0006	Guanidine HCL (GuaHCL)	IB dissolution
D	137037	Guanidinium chloride Emprove® Expert	IB dissolution
D	137030	UREA cryst. Emprove® EXPERT Ph.Eur.,BP,JP,USP,ACS	IB dissolution
D	RES2190D	Dithiothreitol (DTT)	Reduce intra/intermolecular disulfide bonds
D	07604	2- Mercaptoethanol	Reduce intra/intermolecular disulfide bonds
E	104090	Glutathione (reduced) suitable for biopharmaceutical production Emprove® bio Ph.Eur.	Redox reagent
E	G2299	Glutathione (oxidized)	Redox reagent
E	102735	L-Cysteine hydrochloride monohydrate Emprove® ESSENTIAL Ph.Eur.,USP	Redox reagent
E	C5360	L-Cysteine	Redox reagent
E	A4474	L-Arginine	Aggregation inhibitor
E	A4599	L-Arginine monohydrochloride	Aggregation inhibitor
E	107654	Sucrose (saccharose) for density gradient ultracentrifugation suitable for biopharmaceutical production Emprove® bio Ph.Eur.,BP,NF,JP	Protein stabilizer
E	108216	Trehalose	Protein stabilizer
F	106301	CellPrime® r trypsin powder	Precursor maturation
F	106302	CellPrime® r trypsin liquid	Precursor maturation
F	106353	CellPrime® r trypsin STD powder	Precursor maturation
F	106354	CellPrime® r trypsin STD liquid	Precursor maturation

For additional information, visit
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