

## Application Note

# Enabling Rapid Production of Anti-CD7 Recombinant Immunotoxins Using the Constant Systems Platform

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## Accelerating Cell-Free Cancer Therapeutic Development

*E. coli* cell-free systems often produce low amounts of properly folded protein, but adding chaperones can significantly improve both yield and activity. The effectiveness of chaperones depends on the specific protein, so optimization is usually trial and error. Here Simon K Krebs et al in their 2022 International Journal of Molecular Sciences publication show that using this improved prokaryotic system, functional immunotoxins (RIT) were successfully synthesised *in vitro* by these chaperones and shown to bind targets and kill cells. Separately, CHO cell-free systems can also produce these proteins via functional microsomes, but yields are limited due to toxin-related self-inhibition. Self-inhibition aside the CHO cell-free system used in this study yielded enough functional recombinant immunotoxin (RIT) for its *in vitro* characterization.

This rapid, proof-of-concept production relied on robust preparation of high-quality cell extracts (**prokaryotic and eukaryotic**) - enabled by the Constant Systems platform.

## The Challenge

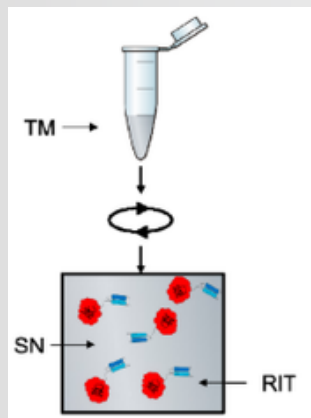
Cell-free protein synthesis requires:

- Highly active, intact translational machinery
- Efficient and reproducible cell disruption
- Preservation of chaperone systems (in *E. coli*)
- Maintenance of microsomal structures (in CHO cells)
- Consistent extract quality for reproducible protein expression

## In this study:

- The prokaryotic system was derived from *Escherichia coli* expressing groEL/groES chaperones
- The eukaryotic system was prepared from Chinese hamster ovary cells, retaining ER-derived microsomes

Extract quality was critical for producing correctly folded, biologically active immunotoxins without the need for downstream purification.



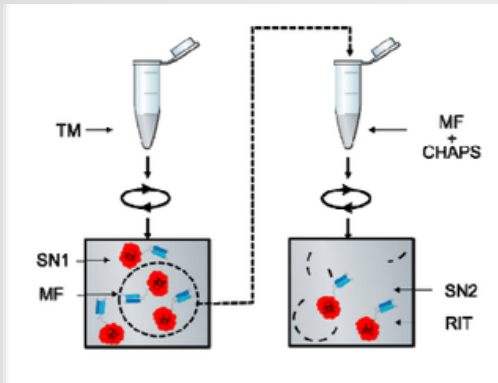
## **E.coli -Based Cell free Protein Synthesis.**

Cells were disrupted with a high-pressure homogenizer Cell Disrupter CF1 (Constant Systems) at 25,000 psi at 2°C.

The supernatant was purified from endogenous amino acids and concentrated. This was shock-frozen in liquid nitrogen and stored at -80°C. for later functional testing.

TM: translation mix  
SN: supernatant  
RIT: recombinant immunotoxin





## CHO cell –Based Cell free Protein Synthesis.

Cells were disrupted using a Constant Systems continuous flow high-pressure homogenizer. The lysate was then clarified by centrifugation to obtain the translation-active supernatant (microsome-containing lysate).

Typical operating parameters: 21kpsi; single pass; 4°C.

This pressure is higher than gentle lysis but lower than bacterial disruption, and it's specifically chosen to break the plasma membrane; break the nucleus and keep ER microsomes intact important for the toxin production system.

- TM: translation mix
- SN1: 1st supernatant
- SN2: 2nd supernatant
- MF: microsomal fraction
- RIT: recombinant immunotoxin

# How the Constant Systems Platform Enabled Success

## 1. High-Quality Cell Extract Preparation

The Constant Systems platform provided controlled mechanical cell disruption, preserving:

- Translational components
- Chaperone activity
- Functional microsomal vesicles
- Structural integrity of cellular machinery all by employing very a specific cell disruption/homogenisation path and active cooling

This directly enabled efficient cell-free protein synthesis, using localised biochemistry on a substrate.

## 2. Reproducible Extract Performance

Consistent upstream processing ensured:

- Batch-to-batch reproducibility
- Reliable immunotoxin expression
- Functional protein folding in both prokaryotic and eukaryotic systems

“We provide the proof-of-concept that cell-free protein synthesis allows for on-demand testing of antibody-toxin conjugate activity in a time-efficient workflow without cell lysis or purification required.” - after harvesting the vital components from cell culture to use in vitro

This study demonstrates:

- First-time production of an active RIT in both E. coli and CHO cell-free systems
- Rapid, on-demand immunotoxin synthesis in vitro
- Elimination of time-intensive purification steps
- Efficient screening of targeted therapeutics

## Constant Systems

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