Setting up an Effective Protein Expression Workflow

Rich Altman[1], Nan Yang[1], Barb Kaboord, Leah Draper, Kokila Rajarathna, Mark L. Barrett, Frederique Boury, Sapna Padmanabhan, Matt McKenna[1] Thermo Fisher Scientific 5781 Van Allen Way, Carlsbad, CA, 92008 thermofisher.com

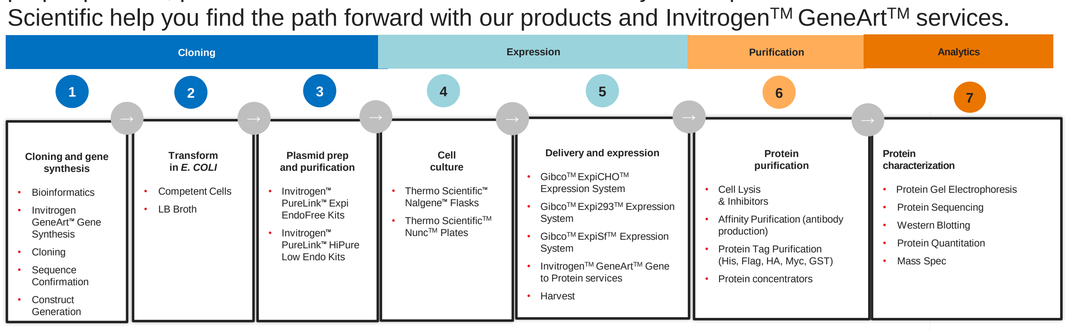
[1]Richard.altman@thermofisher.com, nan.yang@thermofisher.com matt.mckenna@thermofisher.com

Introduction

Transient protein expression technology enables study of gene regulation and protein structure and function. Utilization of recombinant protein expression can also vary widely—from investigation of function in vivo to large-scale production for biotherapeutic drug discovery and structural studies. Using the right protein expression system for your specific application is critical to success. Consider protein solubility, functionality, purification speed, and yield when choosing an expression system. We offer a wide selection of superior mammalian, insect, yeast, bacterial, algal, and cell-free protein expression systems to suit your research needs, backed by trusted brands like Gibco™ and Invitrogen™.

What is the C.E.P.A. workflow

Obtaining the most amount of protein, in the shortest time is the goal of every researcher institution. Optimizing the Cloning, Expression, Purification and Analysis (C.E.P.A.) workflow requires identifying the proper process, products and services to maximize efficiency and improve results. Let Thermo Fisher Scientific help you find the path forward with our products and Invitrogen™ GeneArt™ services.



Choosing the right host system

The choice of the appropriate host system to express your protein of interest is determined by multiple factors such as speed, scale, cost, complexity of protein and government regulations. Thermo Fisher Scientific has multiple host system solutions to meet your expression needs.

Worst → Best

Speed	Transgenics	Plants	Mammalians	Insect	Yeast	Bacteria
Cost	Transgenics	Mammalians	Plants	Insect	Transgenics	Bacteria
Folding	Bacteria	Yeast	Plants	Insect	Transgenics	Mammalians
Glycosylation	Bacteria	Yeast	Plants	Insect	Yeast	Mammalians
Government Regulation	Transgenics	Plants	Insect		Yeast	Bacteria, Mammalians

https://www.brainkart.com/article/Comparing-Expression-Systems_13893/

Maximizing protein expression

Thermo Fisher Scientific has developed the Gibco™ ExpiSf™, Expi293™ and ExpiCHO™ Expression Systems for researchers and facilities needing to rapidly produce high amounts of protein. The Expi Expression Systems offer integrated solutions to deliver the yield, speed, and cost benefits researcher's desire.

ExpiSf™ Expression System

Sf9 cell-based system

Protein yield 3x greater than current platforms
Basic research, gene development

Why?

Insect cells are a cost-effective platform for vaccine production

Other applications:

Intracellular/toxic proteins, multimeric complexes, VLPs, AAV

Expi293™ Expression System

HEK293 cell-based system

Protein yield up to 1 g/L
Structural bio, drug discovery

Why?

Human cells provide native folding and PTMs

Other applications:

mAb screening, reagent/immunogen generation, membrane proteins

ExpiCHO™ Expression System

CHO cell-based system

Protein yield up to 3 g/L
Biologics development

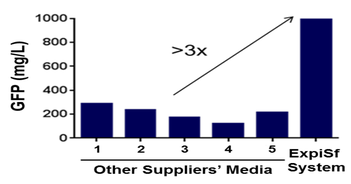
Why?

70% of biologics manufactured in CHO: Get in CHO stay in CHO

Other applications:

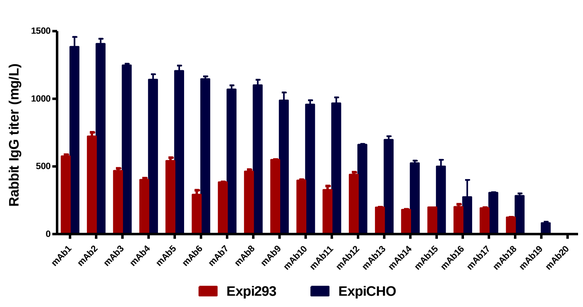
Antibodies, Fc Fusion proteins, Fabs, bispecifics, multispecifics

ExpiSf system outperforms existing insect platforms with **3x higher protein yields**



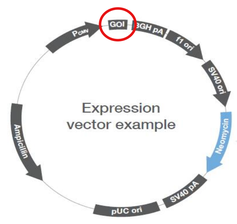
For competitor groups 1-5, Sf9 cells were adapted over multiple passages to each yeastolate-containing competitor media prior to carrying out experiments. For all competitor groups, the classical Sf9 workflow was used (Sf9 cells were seeded at 1e6 cells/mL, incubated overnight, then infected at MOI of 5). For ExpiSf, ExpiSf9 cells were seeded at 5e6 cells/mL, treated with Enhancer, incubated overnight, then infected at MOI of 5. Expression of each protein was done in parallel. Bac-to-Bac™ was used to generate baculovirus for all groups tested. Cells and lysates were harvested 3 days post-infection.

Panel of rabbit monoclonal antibodies tested in Expi293 and ExpiCHO (Max titer protocol) systems



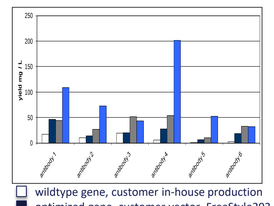
Utilizing our gene optimization and expression services

Advanced gene optimization makes synthesis of very challenging constructs possible as well as providing substantial downstream expression gains. Invitrogen GeneOptimizer™ technology takes into account up to 20 parameters to optimize the mammalian coding region. Thermo Fisher Scientific has developed the ExpiSf, Expi293 and ExpiCHO Expression Systems for researchers and facilities needing to rapidly produce high amounts of protein. The Expi protein expression systems offer integrated solutions to deliver the yield, speed, and cost benefits that researchers desire. Protein Expression services using these systems are available through GeneArt Protein Expression and purification services.



Coding Variables Affecting Expression

- Relative codon usage
- Host codon bias
- Codon pair bias
- GC content
- Direct repeats
- Cryptic acceptor and donor splice sites
- AT-rich sequences
- Poly-G/C sequence
- mRNA secondary and tertiary structures
- Premature poly(A)
- mRNA binding factors
- RNAse cleavage sites
- Internal ribosomal entry sites



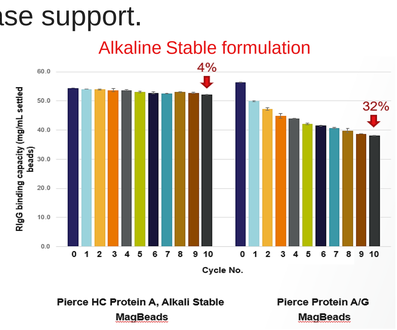
- Optimized HC & LC genes
- IgG kappa leader peptide
- pcDNA3.4
- Expi293 system

Yields improved from 2mg/L to >250mg/L

Optimizing protein purification

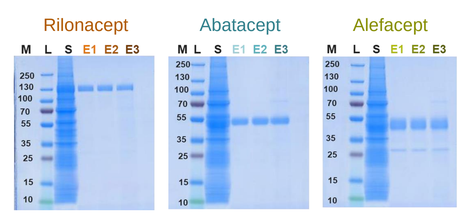
The best clarification and purification workflow is determined by the protein format, scale, yield and level of protein purity needed for downstream applications. Thermo Fisher Scientific™Pierce™ High-Capacity Protein A MagBeads, Alkali Stable, are high-performance magnetic beads for purification of antibodies from cell culture supernatant, ascites, serum, and crude extracts.
These High-Capacity Protein A MagBeads have a high binding capacity (≥ 40 mg HIgG/mL) are Alkaline stable (engineered to withstand standard CIP procedures with 0.1 M NaOH) and compatible with manual and automated workflows (e.g., Thermo Fisher Scientific™ KingFisher™ instruments). The high-affinity Protein A is securely immobilized to magnetic agarose to help prevent leaching of ligand, target antibody, or base support.

Alkaline Stable formulation



Rabbit IgG (RIgG) was bound to Pierce High-Capacity Protein A MagBeads, Alkali Stable, and Pierce Protein A/G Magnetic Agarose, and the beads were eluted and regenerated with 0.1 M NaOH. RIgG binding capacity results over 10 cycles of regeneration are shown. Alkali-stable beads lost only 4% capacity compared with non-alkali-stable protein A/G beads, which lost 32% capacity. Reduction in yield was minimal with the alkali-stable beads, but significantly higher with the non-alkali-stable protein A/G beads.

Purification from unclarified cell culture



Three different Fc-tagged therapeutic proteins were expressed independently in ExpiCHO and secreted into the media. The media containing the overexpressed protein was either centrifuged for 30min at 3000xg and filtered through a 0.2um filter (condition 1), centrifuged at 3000xg for 30min with no filtration (condition 2), or dispensed directly into a deep-well KingFisher plate (condition 3) with no prior treatment. Pierce HC Protein A, Alkali Stable MagBeads were then added directly to the media samples, incubated for 1hr, washed 3X with PBS + 0.025% Tween20, and eluted with 0.2M glycine, pH 2.0. The eluates were immediately neutralized with Tris, pH 8. Samples from each starting cell culture supernatant (S) for each protein and the elutions from the 3 different sample prep conditions (E1-3) were analyzed by SDS-PAGE.

Improving protein analysis

The last step in the CEPA workflow is to verify the size, integrity, and identity of the protein. Researchers use tools such as gel electrophoresis (SDS-PAGE), Western blots, mass spectrometry, chromatography, immunoassays, and cryo- electron microscopy (Cryo-EM) to characterize their proteins. Modernize your Western blotting by implementing the Thermo Fisher Scientific™ iBlot™, iBind™ and iBright™ instruments to analyze publication quality images in a highly-efficient 4hr timeframe.

20minutes

Tanks and high-performance precast gels

Electrophoresis

7minutes

iBlot2 system and ready-to-use consumables

Transfer

3hours

iBind Western Devices

Blot processing

Finished

iBright imager

Detection

Conclusions

Whether you want to study the structure and function of specific proteins or exploit their applications in drug discovery and development, achieving optimal and reliable amounts of recombinant protein is key to success. Various factors are important for achieving high-quality protein expression, from choosing the gene of interest, to characterizing the final purified protein. We are here to support you through every step of the journey. We offer a wide selection of superior and trusted mammalian and insect protein expression workflow solutions backed by a team of professionals, so you can express the right protein faster, at the right volume, and confidently accelerate your research to help improve health outcomes.

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