

6 Steps to Optimize Your Recombinant Antibody Expression

White Paper

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Introduction

Since the mid-1970s, monoclonal antibodies (mAbs) have pioneered discovery and development in the fields of biomedical science, toxicology, biopharmaceutical research, and biological sciences. Monoclonal antibodies have also been proven to be effective therapeutic treatments for diseases such as cancer, autoimmune disorders, and bacterial infections. Traditionally, the process of creating a monoclonal antibody can take anywhere between 6 to 8 months. However, due to the increasing demand of obtaining antibodies faster, researchers have developed alternative methods of producing antibodies. One such method is using commercially available systems and synthetic genes to produce antibodies recombinantly. Recombinant antibodies (rAbs) are monoclonal antibodies that are constructed in vitro from synthetic genes. To begin the process, the heavy and light chains of the antibody are isolated and incorporated into an expression DNA vector. The resulting plasmids are then transfected into a host expression system and expressed. The resulting recombinant antibodies can be used in most of the same applications as traditionally produced monoclonal antibodies. This technology has cut the production time of antibody production time to about 1-2 months, depending on the method.

Recombinant antibodies can be produced in a number of prokaryotic and eukaryotic systems. There are pros and cons to each, but for the purpose of this document, mammalian expression system will be the focal point. Mammalian expression is the ideal system for studying the function of a particular protein in a physiologically relevant environment. This system allows for the highest level of posttranslational processing and functional activity of the protein. Mammalian expression is most commonly used for the production of native antibodies, therapeutic antibodies, and antibodies used in functional cell-based assays. Since antibodies can come in different sizes, formats, folding structure, and more, the optimization strategies for expression must be worked out for each individual antibody. Three aspects are key in recombinant antibody expression: quantity, purity, and functionality. A variety of factors such as poor sequence design and choice of vector, contamination, suboptimal reagents and experimental conditions can lead to protein insolubility, aggregation, or misfolding, which ultimately affect recombinant antibody expression.

In this whitepaper, we will first describe the different formats of recombinant antibodies, how to design your expression vector, transfection methods you could use, and using transient and stable expression methodologies and comparing and contrasting the two. The next sections will discuss several pathways you could manipulate to optimize expression for recombinant antibodies and purification methods that can be used to increase purity and expression. At the end, there is a summary of troubleshooting techniques for various stages of expression in a chart as a reference guide (**Figure 1**).

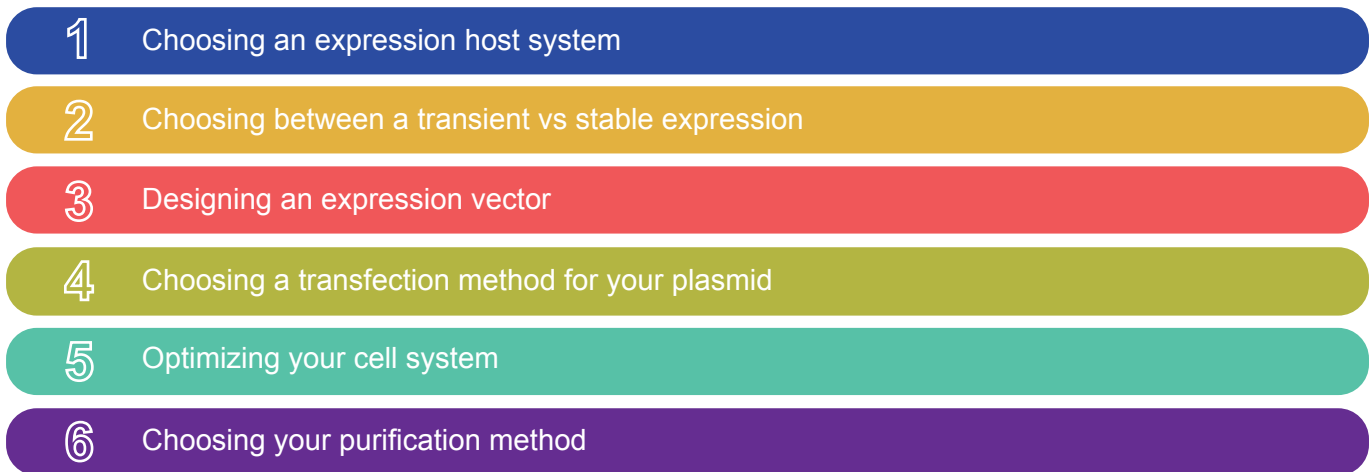


Figure 1: 6 Steps to consider when planning a transient recombinant antibody expression experiment.

Step 1: Choosing Your Expression Host Cell Line

There are a number of different expression systems you can use for recombinant antibody expression, bacterial, yeast, insect, mammalian, plant, and cell free are the most common. This whitepaper will focus on using mammalian host cell lines, since they are more likely to express, properly fold and yield native-like post-translational modifications of secreted antibodies¹. Glycosylation profiles from mammalian-expressed antibodies are also the most consistent with the antibodies observed in vivo and are relatively homogeneous in nature, although there may be minor differences between different hosts^{2,3}. Mammalian cell lines typically used for protein and antibody production are Chinese Hamster Ovary (CHO), NS0, Baby Hamster Kidney (BHK), and Human Embryonic Kidney (HEK293). The most popular choices for transient and stable expression are HEK293 and CHO, respectively. Both cell lines are well documented and have been shown to work excellently for recombinant antibody expression.

- **HEK293 cells** were isolated from human embryonic kidney⁴ and have been used as host for small-scale recombinant protein production for over 2 decades. These cells can be efficiently transfected in suspension at large scale using cost-effective methods such as polyethylenimine (PEI) or calcium-phosphate⁵.
- **CHO cells:** These cells are derived from the ovary of the Chinese hamster. They are the preferred mammalian host for biologics production worldwide, with over 70% approved monoclonal antibodies expressed in these cells.

Step 2: Designing Your Expression Vector

An initial analysis of the primary sequence will tell the researchers if the target will be a challenge to produce. There are several bioinformatics tools available that can help you with your vector design.

One that **GenScript** offers is **GenSmart™ Smart**. GenScript - the world's leading provider of molecular biology services – has developed **GenSmart™ Design**: a smart construct design interface to make building constructs easy for researchers of all experience levels across the globe. **GenSmart™ Design** is developed based on a part-driven design philosophy and backed up with our proprietary algorithm that integrates all you need to design a DNA construct. GenScript also offers a codon optimization tool named **OptimumGene™**. OptimumGene™ Gene Design system is a proprietary gene optimization technology that can alter both naturally occurring and recombinant gene sequences to achieve the highest possible levels of productivity in any given expression system. The OptimumGene™ algorithm takes into consideration a variety of critical factors involved in different stages of protein expression, such as codon adaptability, mRNA structure, and various cis-elements in transcription and translation. For more information on either of these services please visit www.genscript.com.

Once you have designed your target and chosen your expression vector, the next step is to design a cloning scheme. Typically, many researchers these days tend to adopt parallelization strategies to facilitate high throughput cloning and expression. For example, strategies like Ligation Independent Cloning (LIC) or recombination-based cloning (In-Fusion Cloning) will allow the generation of multiple expression constructs at once, for parallel expression testing. While LIC is friendlier, because it adds minimum vector residues and is perceived as more friendly to crystallography, recombination-based cloning is also a very efficient method. Ligation Independent Cloning (LIC) approach utilizes the 3' → 5' exonuclease activity of T4 DNA Polymerase to create overhangs with complementarity between the vector and insert. LIC employs relatively longer overhangs which form stable joints between fragments, allowing for transformation without ligation step. Owing to a dual polymerase/exonuclease function, T4 DNA polymerase can create overhangs of varying lengths based on a defined sequence. The annealed vector is repaired during replication. Recombination-based cloning method such as In-Fusion cloning, is designed for fast, directional cloning of one or more fragments of DNA into any vector. The method typically uses PCR-generated DNA fragments and linearized vectors that contain a 15bp overlap at their ends. This region is responsible for the recombination. With the advent and affordability of synthetic genes, many scientists choose to have their genes designed, synthesized and sub cloned into an expression vector.

Step 3: Delivering Your Plasmid

You will next need to determine the delivery method for your plasmid. There are several different methods you can use to introduce a gene of interest into mammalian cells.

- **Polyethylenimine (PEI) or Calcium Phosphate:** This method is the tried and true version of transfection, which utilizes chemicals such as polyethylenimine (PEI) or calcium-phosphate^{4,6} to incorporate the plasmid into the cells. PEI is a stable cationic polymer that condenses DNA into positively charged particles that bind to the anionic cell surface, where it is later endocytosed into the cell⁶. Calcium-phosphate works in a similar method as PEI, but can have variability due to its sensitivity to slight changes in pH, temperature, and buffer salt concentrations, and can be cytotoxic to many types of cell cultures⁴.
- **Electroporation:** Electroporation is the process of using an electrical field on cell cultures in order to increase the permeability of the cell membrane to all DNA to be introduced into the cell⁷. This process is highly effective for transfecting cells in suspension or adherent cultures, but a downside to electroporation is that requires expensive equipment and also causes physical damage to the cells⁸.
- **Recombinant Viral Transduction:** Viral transduction is usually the preferred choice for cell types that are difficult to transfect or non-dividing cell types. Retroviral, Lentiviral (a more complex retroviral), or adenoviral expression systems are the most common expression systems used. It allows for stable integration of the transgene, which leads to the gene of interest being continuously expressed over repeated cell divisions. One key feature of retroviral and lentiviral transduction is that they produce replication defective particles, which allows for the delivery of the desired sequence, without continued viral replication.

All of these methods have been shown to successfully induce gene expression and have well established benefits, but sometimes troubleshooting and optimizing the system could cost a research valuable time. With the advent and affordability of synthetic genes, many scientists choose to have their genes designed, synthesized and sub cloned into an expression vector.

GenScript offers a comprehensive gene synthesis portfolio that can assist you in every stage of gene synthesis, plasmid preparation, molecular cloning, and mutagenesis. Please visit:
<https://www.genscript.com/molecular-biology-service.html>

Step 4: Choosing between Transient Vs Stable Expression

Mammalian expression experiments can be conducted either transiently or stably in cell lines in order to express your protein or antibody of interest. Both these transfection methods involve getting the foreign (target) gene into the cells. In transiently transfected cells, the foreign DNA does not integrate into the host genome and as such it does not replicate and is eventually lost through cycles of cell division over several days. Transient transfection is good if you have relatively small yield requirements for recombinant antibody (rAb) and if you need it fast (**Table 1**). Transient gene expression results in short term recombinant Ab production, typically 6-10 days from

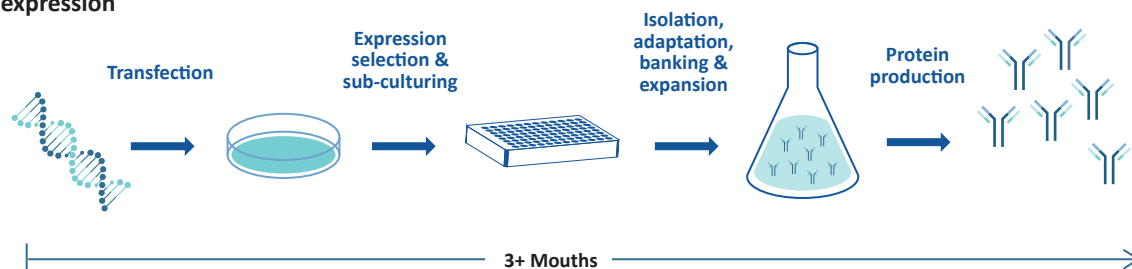
the point of DNA transfection. HEK293 cells normally achieve higher transfection rates and higher yields with PEI, which is inexpensive and resulting in lower overall production costs. Transient expression is ideal for rapid protein production, allowing researchers the ability to use any mammalian expression vector, and to generate data quickly.

Approach	Pros	Cons	Application
Transient	Quickest timeline	Moderate titer	Reagent and early drug discovery
Stable pool	Higher titer than transient	Pool will decay during repeated production	Used to increase transient expression yield as a reagent.
Stable cell line	Stable production	Long setup time	Large scale production

Table 1: Pros and cons between the types of expression methods you can use for recombinant antibody production.

In stably transfected cells, the foreign gene becomes part of the host genome and is therefore replicated. Stable transfection also begins transiently but through a process of careful selection and amplification, stable clones are generated. Descendants of these transfected cells, also express the foreign gene, resulting in a stably transfected cell line. Because the stable transfection of cells is a longer and more arduous process, it is practical for rAb production on larger scales. During the initial selection process when research quantities of rAbs are sufficient (10-1000 mg), rapid methods of rAb production are required and so large scale transient expression is routinely used. Stable cell lines can also be used over again in many experiments and is ideally suited for a long experimental time course. Since the expression vector has a selection marker (such as an antibiotic) that is added to the media and could cause stress unto the cells, it is recommended to perform a dose-response curve to determine the optimal concentration.

Stable expression



Transient expression

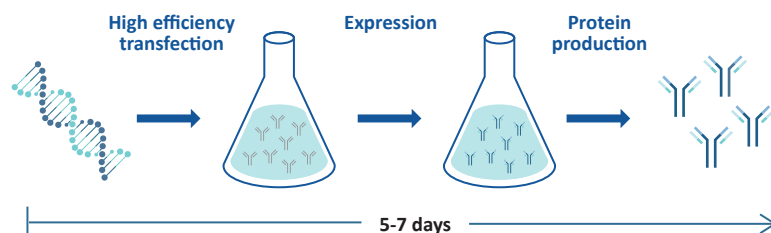


Figure 2: Depiction of the workflow and timeline between transient and stable expression.

Step 5: Optimizing Your Cell System

Transient transfection protocols are fairly similar to one another and have the same basic components. In order to perform a successful experiment, it is recommended to begin with maintaining healthy cells. Cells require a number of components (glucose, nutrients, serum, vitamins, etc.) to grow healthy and be susceptible to transfection.

One major pathway we recommend tinkering with is the apoptotic pathway. Apoptosis is the process of programmed cell death. It is a highly regulated pathway that can be induced by two ways, 1) an intrinsic (internal) pathway trigger such as cellular stress or 2) an extrinsic (external) pathway trigger such as signals from other cells⁹. Apoptosis in cell culture is usually caused by nutrient depletion and/or metabolite accumulation and can be controlled using different methods. One method is to optimize your media by controlling the accumulation of metabolites by using components to reduce glutamine or bicarbonate. This helps to modulate the amounts of ammonia and carbon dioxide, respectively, in your system¹⁰. Another method for tackling apoptosis is manipulating cells in favor of cell survival to develop resistance to apoptosis. Some studies have shown that cells developing resistance to apoptosis by altering gene expression to favor cell survival or by manipulating the DNA repair pathway, can increase growth potential of cells¹¹. Manipulation of the apoptosis pathway is a very potent way to help optimize your cell system for antibody expression.

In order to optimize transient antibody expression in cell lines, you can use different components or methods to alter the proliferation or cell growth pathway. Most cell lines require the complete growth media to grow. However, there are some additional media components that you can use, such as hormones, growth factors, and signaling substances, that have been shown to help optimize your antibody expression. One example of a growth factor, LONG R3 IGF-I, is an engineered peptide that has been shown to enhance cell viability over a 12 day experiment in some cell lines such as CHO and HEK 29312,13. In order to test which type of component would be good for your cell system, you will need to do a media analysis test. This will help you determine which combination of additives help to increase your antibody expression, with minimal changes to your cell viability. You can also add additives to your cell culture to help optimize expression. An example of an additive is histone deacetylase inhibitors that de-condense your chromatin and increase your transcriptional activity. Proprietary feed solutions such as HyClone Cell Boost®, can also increase your antibody expression by supplementing essential components in your media that naturally become depleted during antibody production¹². Overexpression of different proteins (Aven, Bcl-2, Htert, are a few), can actually increase the proliferation and survival of some cell lines^{14,15}.

GenScript has used different components that regulate cell growth for our **High Density (HD) Transient Expression HEK 293** cell lines and has shown that using the components together at the same percentage, increased cell growth as compared to using them individually (**Figure 3**).

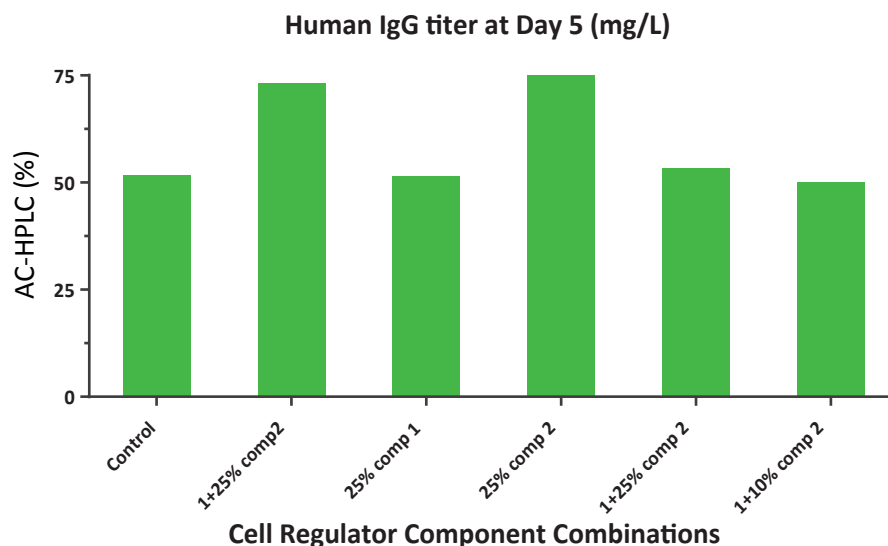
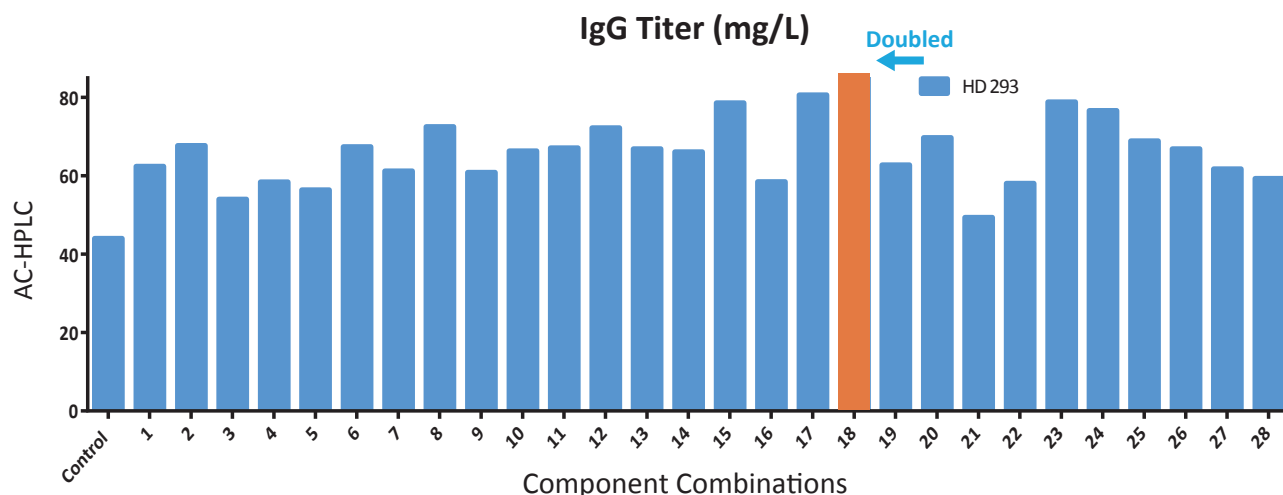


Figure 3: Optimization of recombinant antibody expression using different cell growth regulators in specialized GenScript owned High Density (HD) 293 cells. The following results show that Component 1 does not work alone and Component 2 can improve antibody expression by 85% when added to cells.

Another major pathway that can be adjusted in order to increase protein expression is the cell metabolism pathways. Cellular metabolism pathways are interrelated networks and the characteristics of these pathways can help with medium optimization. An example is the glycolysis pathway and the TCA cycle. If you have a cell line that tends to produce lactate, it would be beneficial to use components or chemicals that will lead to a metabolic flux towards the TCA cycle. Another example is that metabolism components can also impact antibody production and biomass formation. If a cell line has a high growth rate but low productivity, limiting the metabolism rates could force the cells to work on protein production instead of biomass¹⁶.

GenScript has tried several metabolic boosters such as energy boosters, stress releasers, and protein folding enhancers to optimize our transient expression production (**Figure 4**). These different boosters need to be tested individually, in combination with one another, and at different amounts within your system. Each of them can act differently upon the cells, so it is good to test them initially in small amounts and in different combinations.



Component Cocktail of Sample 18	Component 2	Component 4	Component 5	Component 6
Ratio (%)	10	10	10	10
Function	Cell cycle regulator	Protein folding enhancer	Growth stress releaser	Energy booster

Figure 4: Optimization of transient expression using a metabolic, cell cycle and protein folding pathway modifications in specialized HD-293 cells. Results: Using 28 different pathway modification combinations, Sample 18 proved to be the most effective chemical cocktail at increasing protein expression. The samples were collect on day 5 after transfection.

Some studies have shown that in some systems, introducing a gene that helps to promote cell survival by circumventing the stress response pathway¹⁷. The cellular stress pathway is a cell’s response to stressors, such as temperature changes, toxins, environment stressors, damage, etc. In one paper, scientists showed that by inducing a small amount of an external stressor unto cells to induce a stress response can actually lead to a several fold increase in difficult to express proteins¹⁸. Another system that can be manipulated to help increase protein expression is protein folding. Protein folding is the physical process by which a protein acquires its native conformation that usually helps with its biological function. Proteins require proper folding to do their biological function, especially protein therapeutics that require proper folding to achieve the three-dimensional conformation in order to be functional. Also, there are several diseases that are caused by protein misfolding, reiterating its importance not only in research but in biology. Studies have shown that facilitating the proper protein folding can help increase protein production¹⁹.

GenScript has a comprehensive protein expression platform. All services contain a similar workflow but are adjusted as necessary for each service.

The general workflow is as followed:

1. Each service begins with a customer supplied protein sequence or customer-supplied DNA vector.
2. GenScript then performs codon optimization using OptimumGene™, GenScript's PSO-based proprietary gene optimization technology. Followed by gene synthesis, plasmid preparation, and construct generation.
3. The gene of interest is then subcloned into GenScript proprietary expression plasmid.
4. The expression plasmid is then transfected into the expression system of choice. The cells are then cultured, harvested, and the protein is collected.
5. Then there is one-step of purification. The purified protein then undergoes our strict quality control process.
6. Lastly, the purified protein, DNA construct (upon request), and QC results are delivered to the customer.

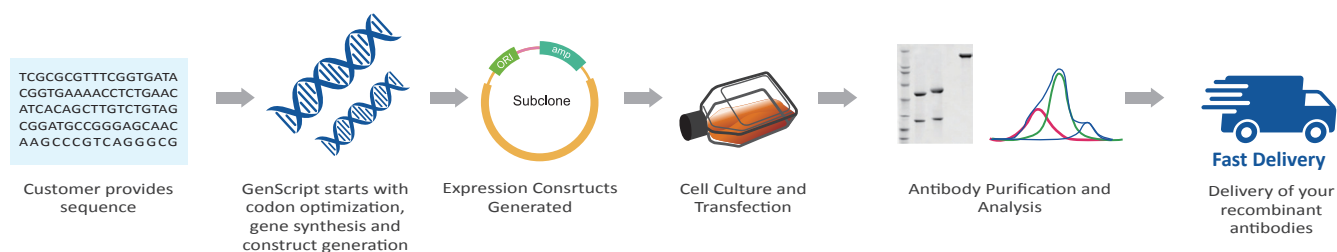


Figure 5: GenScript's one-stop-shop platform from gene synthesis to purified recombinant antibody.

Step 6: Purification Techniques

Another strategy to improve recombinant antibody production is to improve your purification method. Recombinant antibodies can be purified manually or by using a chromatography system. Depending upon application and purity requirements, the purification steps can be planned out. If the antibody expression is robust, a 1-step affinity purification step will yield sufficient antibody and adequate purity for a variety of applications. If further purification is required, it can be typically followed with Size Exclusion Chromatography (SEC). Ion Exchange (IEX) Chromatography and Hydrophobic Interaction Chromatography (HIC) can be used if further polishing is required.

- **Affinity chromatography**, separates proteins on the basis of a reversible interaction between protein and a specific ligand that is coupled to a chromatography matrix. The technique offers high selectivity, high resolution, and high capacity for the protein of interest. Recovery of purified material is generally very high.

- **Size Exclusion Chromatography (SEC)**, also called Gel Filtration (GF) chromatography. SEC separates molecules according to differences in size as they pass through the resin. The goal can be to isolate one or to analyze the molecular-weight distribution in the sample. SEC allows the user to increase purity as well as homogeneity of the antibody sample. Unlike in Ion Exchange or Affinity Chromatography, molecules do not bind to the chromatography medium.
- **Ion Exchange (IEX) Chromatography**, separates antibodies on the basis of differences in their net surface charge. It takes advantage of the fact that the relationship between net surface charge and pH is unique for every antibody. Depending upon their charge properties, antibodies exhibit different degrees of interaction with charged chromatography media. Depending on the antibodies isoelectric point (pI), either anion-exchange or cation-exchange matrices are used in low salt concentrations to bind the target proteins while contaminating proteins bind relatively weakly. As the salt concentration is increased, the interactions of charged groups on protein surface becomes weaker and eventually all proteins elute at a characteristic salt concentration range and can be separated.
- **Hydrophobic Interaction Chromatography (HIC)**: HIC separates antibodies according to differences in their surface hydrophobicity by utilizing a reversible interaction between these proteins and the hydrophobic surface of a medium. An inverse gradient from high salt to low salt concentration is applied to the column. The elevated salt concentration enhances interaction between the hydrophobic components of the sample and the chromatography medium. During separation, samples are purified and eluted in smaller volumes, thereby concentrating the sample so that it can go directly to gel filtration or to an ion exchange separation (after a buffer exchange). HIC can be used for capture, intermediate purification or polishing steps in a purification protocol.

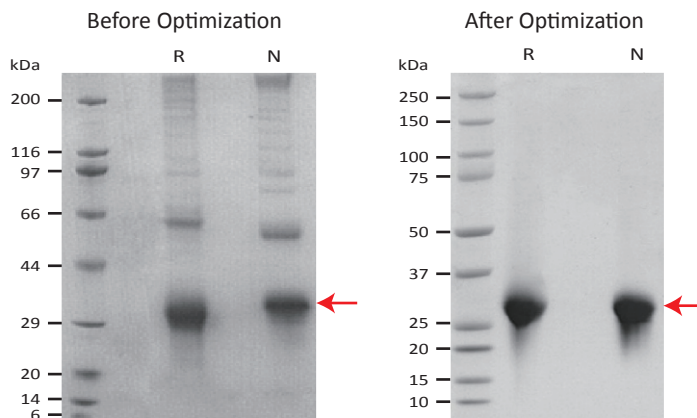


Figure 6: SDS-PAGE analysis of His-tagged proteins purified after one-step purification using different columns. Protein X is indicated with red arrows. The corresponding expression levels are listed in the following table.

(R) Reducing condition
(N) Non-reducing condition

Troubleshooting:

Stage	Problem	Comments
Expression	No Expression	<ul style="list-style-type: none"> Check to make sure DNA sequence is OK
	Low Expression	<ul style="list-style-type: none"> Codon optimization to enhance expression Optimize expression conditions (media, lower induction temperature, etc.)
	Truncated Expression	<ul style="list-style-type: none"> Ensure DNA sequence is OK and that there are not premature stop codons Try induction at lower temperature
Purification	Wrong protein/antibody purified	<ul style="list-style-type: none"> Check DNA to ensure construct integrity
	Protein/Antibody elutes with contaminants Additional Purity Needed	<ul style="list-style-type: none"> Binding and wash conditions need to be more stringent – include up to 20 mM imidazole in binding and wash buffers Column too large – Reduce resin amount Contaminants associated with tagged protein – add reducing agent such as β-ME to reduce disulfide bond formation
	Protein/Antibody precipitates	<ul style="list-style-type: none"> Perform additional purification steps such as Ion-Exchange Chromatography Maintain low concentrations <1 mg/mL Maintain adequate reduced state >5 mM DTT to prevent oxidation Maintain high salt concentration 500 mM and add glycerol >10% Add arginine in the range of 50 mM-500 mM Add mild non-denaturing detergent such as 0.1% β-octylglucoside
	Protein/Antibody elutes during wash steps	<ul style="list-style-type: none"> Perform purification at room temperature Lower imidazole concentration in wash buffer
	Protein/Antibody does not elute	<ul style="list-style-type: none"> Reduce stringency of wash buffer Elution conditions may be too mild – elute with pH or elution buffer with step-gradient to determine optimal elution conditions Protein maybe in aggregate (oligomer/multimer) form – Elute under denaturing conditions Protein has precipitated in column – perform binding and elution in batch format to avoid high protein concentration locally

Table 2: Troubleshooting table to assist at different stages of transient expression experiments.

Summary:

We have summarized various strategies that can improve your protein expression. These strategies, while not comprehensive, are of considerable importance for achieving reliable and high-level protein expression for your research. Please see the following information to learn how GenScript' service can help increase your recombinant antibody yields.

High Density Transient Expression System:

Keeping customer needs in mind, GenScript has launched a new, High Density (HD) Transient Expression service for the high-titer production of your recombinant antibodies and proteins in either CHO or HEK293 cells. Using HD mammalian cell culture, this HD Transient Expression service can deliver unprecedented improvements in functional protein/rAb yields compared to regular Transient Expression services. Using this new service, GenScript scientists can deliver gram quantities of intracellular and secreted proteins (including antibodies) for your functional, structural and therapeutic studies.

Key features of HD Transient Expression Service

- **HD-HEK** and **HD-CHO** for choice of HEK293 or CHO expression
- **Expert vector design** at the molecular level to optimize antibody expression
- Extensive **experience** with **difficult antibody** formats.
- Yield improvements ranging up to **100 fold**
- **Shortened turnaround** time (sequence to protein/antibody in as little as **10 weeks**)
- Up to **3 g/L rAb** titer possible
- Learn more at: <https://www.genscript.com/high-density-transient-expression.html>

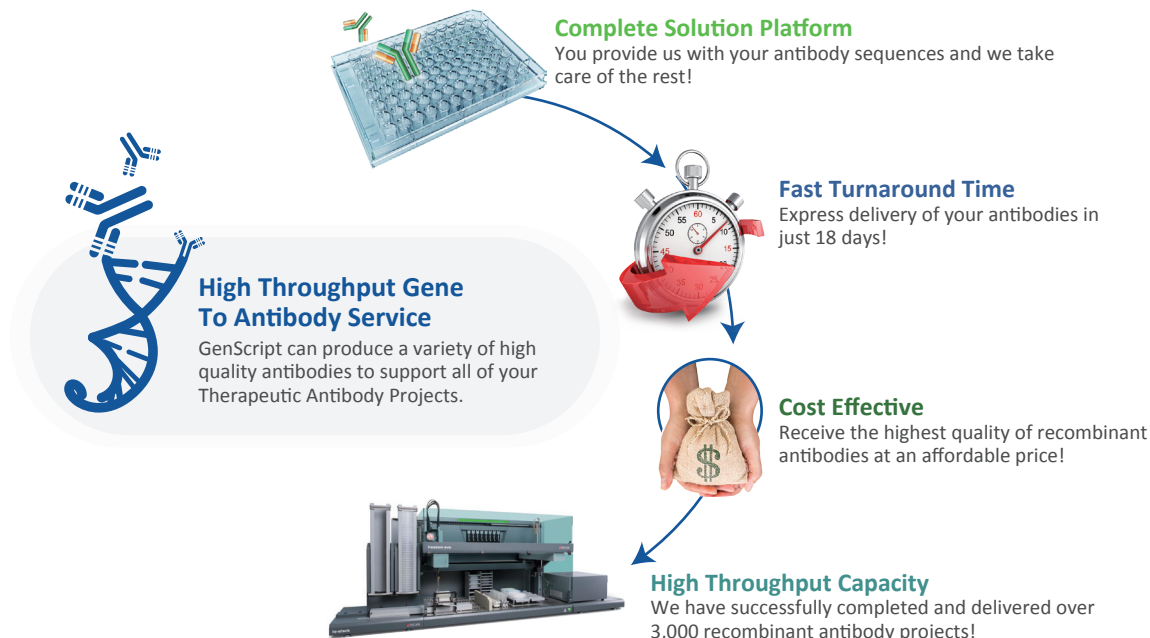
References:

1. Dalton, A. C. & Barton, W. A. Over-expression of secreted proteins from mammalian cell lines. *Protein Sci.* **23**, 517–25 (2014).
2. Jenkins, N., Meleady, P., Tyther, R. & Murphy, L. Strategies for analysing and improving the expression and quality of recombinant proteins made in mammalian cells. *Biotechnol. Appl. Biochem.* **53**, 73–83 (2009).
3. Chusainow, J. et al. A study of monoclonal antibody-producing CHO cell lines: what makes a stable high producer? *Biotechnol. Bioeng.* **102**, 1182–96 (2009)
4. Graham, F. L. & van der Eb, A. J. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–67 (1973).
5. Durocher, Y., Perret, S. & Kamen, A. High-level and high-throughput recombinant protein production by transient transfection of suspension-growing human 293-EBNA1 cells. *Nucleic Acids Res.* **30**, E9 (2002).
6. Longo, P. A., Kavran, J. M., Kim, M.-S. & Leahy, D. J. Transient mammalian cell transfection with polyethylenimine (PEI). *Methods Enzymol.* **529**, 227–40 (2013).
7. Neumann, E., Schaefer-Ridder, M., Wang, Y. & Hofschneider, P. H. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* **1**, 841–5 (1982).
8. Kim, T. K. & Eberwine, J. H. Mammalian cell transfection: the present and the future. *Anal. Bioanal. Chem.* **397**, 3173–8 (2010).
9. Elmore, S. Apoptosis: A Review of Programmed Cell Death. *Toxicol. Pathol.* **35**, 495–516 (2007).

10. What media components can reduce apoptosis and increase cell viability beyond critical density? - Cell Culture Dish - Cell Culture Dish. Available at: https://cellculturedish.com/ask_the_expert/what-media-components-can-reduce-apoptosis-and-increase-cell-viability-beyond-critical-density/. (Accessed: 20th July 2018)
11. Pritchard, D. E. et al. Resistance to apoptosis, increased growth potential, and altered gene expression in cells that survived genotoxic hexavalent chromium [Cr(VI)] exposure. *Mol. Cell. Biochem.* **279**, 169 (2005).
12. Almo, S. C. & Love, J. D. Better and faster: improvements and optimization for mammalian recombinant protein production. *Curr. Opin. Struct. Biol.* **26**, 39–43 (2014).
13. Voorhamme, D. & Yandell, C. A. LONG R3IGF-I as a more potent alternative to insulin in serum-free culture of HEK293 cells. *Mol. Biotechnol.* **34**, 201–4 (2006).
14. Turturici, G. et al. Hsp70 is required for optimal cell proliferation in mouse A6 mesoangioblast stem cells. *Biochem. J* **421**, 193–200 (2009).
15. Reuschel, E. L. et al. REDD1 Is Essential for Optimal T Cell Proliferation and Survival. *PLoS One* **10**, e0136323 (2015).
16. Can you explain how cell metabolism characteristics can be used to optimize media in CHO cells? - Cell Culture Dish - Cell Culture Dish. Available at: https://cellculturedish.com/ask_the_expert/can-you-explain-how-cell-metabolism-characteristics-can-be-used-to-optimize-media-in-cho-cells/. (Accessed: 20th July 2018)
17. Malik, A. et al. Optimization of expression and purification of HSPA6 protein from *Camelus dromedarius* in *E. coli*. *Saudi J. Biol. Sci.* **23**, 410–419 (2016).
18. Chhetri, G., Kalita, P. & Tripathi, T. An efficient protocol to enhance recombinant protein expression using ethanol in *Escherichia coli*. *MethodsX* **2**, 385–391 (2015).
19. Hopkins, R. F., Wall, V. E. & Esposito, D. Optimizing Transient Recombinant Protein Expression in Mammalian Cells. in *Methods in molecular biology (Clifton, N.J.)* **801**, 251–268 (2012).

High Throughput-Recombinant Antibody Platform

GenScript's Premier High Throughput Gene to Antibody Service



Service Details

Starting material	Expression	Amount	Price	Timeline	Deliverable
Antibody Sequence (Minimum 4 samples)	Mammalian	10 mL of Supernatant (SC1945)	\$339/Ab	10 Business Days	<ul style="list-style-type: none"> 10 mL of crude antibody supernatant QC data
		100 µg (SC1796)	\$499/Ab	18 Business Days	<ul style="list-style-type: none"> 1-step affinity purified antibody as specified QC data
		1 mg (SC1797)	\$799/Ab		
		Customized Service (SC1946)			

- Project can begin from HTP gene synthesis or customer-supplied DNA but Antibody sequences are required for project evaluation in both cases
- Turnaround time does NOT include shipping details. Please add approximately 3-5 days to account for shipping onto the dates listed.
- Pricing applicable to human IgG1, human IgG4, and mouse IgG2 a/b; Kappa (κ)/lambda (λ) light chains
- Quality Control (QC) data includes: A280 for antibody concentration and SDS-PAGE for purity assessment. If further analysis is required, GenScript will also perform western blotting.

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