4 Strategies for Boosting Transient Protein Expression

Application Note
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Introduction

Recombinant proteins are essential tools in basic and applied biomedical research. Using the power of recombinant DNA (rDNA) technology, production of recombinant proteins in desired quantities and purities for a variety of applications has become more widespread. Moreover, this technology enables scientists to engineer proteins for specific study objectives. Since every protein has unique structural and functional characteristics, tailored protocols must be developed to optimize the expression and purification of each individual protein. In this application note, we will describe key factors affecting transient protein expression yield in a mammalian system and discuss essential strategies to modify them in order to obtain functional proteins in desired quantity and purity.

Three aspects are key in recombinant protein 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 protein expression.

Strategy 1: Designing an Expression Vector

The strategy to avoid many of the above-mentioned problems is to design the best coding sequence and choose the most appropriate expression vector for your specific downstream application. Using advanced codon optimization algorithms, such as OptimumGene™, and expression vector selection guides, such as GenScript's GenSmart™ Design Tool, before you start your expression can save you effort, time, and money. Adopting the following strategies can also help you avoid or address issues with suboptimal protein expression regardless of your specific sequence or expression system.

Strategy 2: Increasing Protein Solubility

Low protein solubility is a key factor in obtaining a low yield in recombinant expression. Before redesigning your sequence and expression vector, we recommend you to first manipulate some of the common expression condition parameters in order to enhance protein solubility:

- **Temperature**: For HEK293 and other mammalian cell lines, it is recommended to grow cells at 37°C in a 5% CO₂ incubator. However, some studies have shown that reducing the temperature from 37°C to 33°C for 24 hours after transfection can increase protein expression in HEK293 cells. Although it is known that lower temperatures can reduce the growth rate of cells, some studies have shown that lower temperatures can actually improve cellular productivity of the system for protein production. This means that it can lead to an increase of recombinant protein yields and reduce degradation of proteolytically-sensitive proteins.
• **Growth Medium:** The most common method to cultivate cells for recombinant protein expression is through batch culture. Therefore, all nutrients that are required for cell growth must be supplied from the beginning by inclusion in the growth medium. Although different vendors offer similar base media, there are subtle differences between them that might affect the growth of your specific expression cell line. Also, make sure to optimize the concentration, pH, type, and even batch or lot number of the external components, such as serum, you add to growth media.

• **Media Supplements:** In some cases, it is recommended to add a specific agent to growth medium to enhance protein expression. One example is adding histone deacetylase inhibitors to decondense chromatin and increase the transcriptional activity of integrated genes. Another example is co-transfection of growth factors and cell cycle regulators, such as acidic fibroblast growth factors that have also been shown to increase protein expression. Lastly, adding a particular growth factor to growth medium can help increase protein yields. For example, the LONG R3 IGF-I engineered peptide has shown to have enhancing properties like doubling the cell viability over 12-day experiments in cell lines such as CHO, HEK293, and PERK.C6 compared to traditional insulin additives.

**Strategy 3: Utilizing a Fusion Partner**

Another strategy to optimize the expression of low-yield proteins is the gene fusion partner technology. This strategy utilizes promoters, enhances, tags, and other fusion elements to increase the solubility of your protein. Protein tags are short peptide sequences that are grafted onto a recombinant protein and are usually removed by enzymatic means or by chemical agents at the end of protein expression. There are several common tags that one can use in one’s experiment to enhance protein expression. You can refer to Table 1 for a list of the common types of protein tags and their advantages and disadvantages.

<table>
<thead>
<tr>
<th>Tag</th>
<th>~MW [kDa]</th>
<th>Affinity matrix</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
</table>
| His | ≤1       | Nickel         | • Universally used  
• Small in size  
• Works with native and denaturing conditions | • Usually requires more than 1-step elution  
• Not entirely innocuous |
| Flag | 1        | Anti-Flag      | • High Purity | • Expensive  
• Elusion can be occasionally problematic |
| Fc  | 25       | Protein A      | • Used for large proteins  
• Simple detection with ELISA kits  
• Simple affinity purification by A, G, and L affinity purification resins | • Nonspecific binding is a possibility  
• Cleavage step necessary |
| GST | 26       | Glutathione    | • Easy elution  
• Solubilizing tag | • Tendency to dimerize |

**Table 1:** List of Commonly used tags

Selection of the right tag for a specific expression system depends on a variety of factors. Top criteria include cost, binding and regenerative capacity of affinity resin, condition of purification buffer, and the overall objective of the experiment. For example, if your goal is to obtain a very high yield, you need to use solubility tags like GST, which have strong translational initiation signals to drive high level of expression. However, if you need lower level of expression, then we recommend using a more stringent epitope tag such as His tag. A clear demonstration of this example is shown in Figure 1, where protein aggregation had affected the expression of protein X. In order to resolve this issue, senior scientists at GenScript used different protein tags to reduce protein aggregation and obtain protein X at a higher yield.
At the end of the process, some of these partners are left intact, but the majority of them are cleaved off of the target protein. When adopting this approach, refer to Table 2 for some considerations.

1. If the fusion partner is too large and may interfere with the natural function of the target protein, it must be cleaved off.

2. Removal of a C-terminal tag can be tricky because oftentimes the extraneous amino acids are left behind from the protease cleavage site.

3. Cleavage itself can be an issue due to steric hindrance. This can be resolved by including a linker region between the proteolytic cleavage site and the fusion partner.

4. Some fusion proteins do not bind efficiently to resin and even when they do, yields can be suboptimal. To address possible target protein precipitation post-cleavage, consider optimizing protein purification conditions or change your affinity purification strategy (e.g., using His tag instead).

Table 2: Points to consider when using a protein tag or another type of fusion partner

GenScript offers services to troubleshoot protein projects by using a variety of methods, such as optimizing solubility conditions and manipulating the fusion partner strategy. As shown in figures 2 and 3, GenScript’s High Density Transient Expression service, was able to improve the yield of the TGF-B1 protein and the full-length human AMH protein by employing the fusion partner optimization strategy. This service employs a proprietary transient mammalian expression technology to modify cellular pathways to achieve optimal protein/antibody expression yields (up to 3 g/L). Our scientists can also optimize expression protocols and growth media to maximize cell longevity, for guaranteed gram-level recombinant protein/antibody expression projects. GenScript’s High Density Transient Expression service is able to deliver gram quantities of intracellular and secreted proteins for your functional, structural and therapeutic studies.
Strategy 4: Improving Purification

The last essential strategy is on improving your purity method. Recombinant proteins can be purified manually or by using a chromatography system. Depending upon you desired application and purity requirements, different purification methods can be planned out. If protein expression is robust, a 1-step affinity purification method will yield sufficient protein and adequate purity for a variety of downstream applications. If further purification is required, affinity purification can be typically followed by Size Exclusion Chromatography (SEC). For further purification, an Ion Exchange (IEX) Chromatography can be used.

- **Affinity Chromatography (AC)** 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. With this method, the recovery of purified material is generally very high.

- **Size Exclusion Chromatography (SEC)**, also called Gel Filtration (GF) chromatography, separates molecules according to differences in size as they pass through the resin. The goal can be to isolate one or more proteins in a mixture, or to analyze the molecular-weight distribution in a sample. SEC allows the user to increase purity as well as homogeneity of the protein sample since various oligomeric states of the same protein can be resolved successfully. Unlike IEX or AC, in SEC molecules do not bind to the chromatography medium.

GenScript’s High Density Transient Expression service employs a proprietary transient mammalian expression technology to modify cellular pathways to achieve optimal protein/antibody expression yields (up to 3 g/L). Our scientists can also optimize expression protocols and growth media to maximize cell longevity, for guaranteed gram-level recombinant protein/antibody expression projects.
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- **Ion Exchange (IEX) Chromatography**, separates proteins 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 protein. Depending upon their charge properties, proteins exhibit different degrees of interaction with charged chromatography media. Depending on the protein’s isoelectric point (pI), either anion-exchange or cation-exchange matrices are used in low salt concentrations to bind 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.

If you need to improve your purity method, we first recommend changing your purification column. As shown in Figure 4, when using a nickel column, the purity of the protein of interest was about 65%. However, after using the GenScript’s His-column product, we were able to improve the protein purity to about 90%. GenScript’s High Density Transient Expression service, is able to provide clients with a minimum of 90% purity for their protein projects and is able to customize the purity and endotoxin level based on your specifications.

![Figure 4: SDS-PAGE analysis of His-tagged proteins purified after one-step purification using different columns.](image)

**Summary**

In this application note, we reviewed a variety of essential strategies that can improve your protein expression. These strategies, while not comprehensive, are of considerable importance for obtaining reliable and functional recombinant proteins at high yields. Please see the following information to learn how GenScript’ service can help increase your recombinant protein yields.
GenScript’s High Density Transient Expression System

To make research easy for our customers, 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 cell lines. Using HD mammalian cell culture, this HD Transient Expression service can deliver unprecedented improvements in functional recombinant protein yields compared to regular transient expression services. Using this new service, GenScript’s scientists can deliver gram quantities of intracellular and secreted proteins (including antibodies) for your functional, structural and therapeutic studies.

Key features of GenScript’s 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 protein expression
- Extensive experience with difficult protein formats
- Yield improvements up to 100 folds
- Shortened turnaround time; sequence to protein in as few as 10 weeks
- Experts with 15 years of experience and a 95% success rate on projects

References


