Application Note

Genomics

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Quality Control of Protein Expression and Purification Steps with the Agilent ProteoAnalyzer System

Author

Whitney Pike,
Agilent Technologies, Inc.

Abstract

The production of high-quality recombinant proteins is essential for various industries. The Agilent ProteoAnalyzer system, an automated quality control instrument, provides precise measurements of diverse protein samples. This application note demonstrates how the system can be used throughout the protein expression and purification process for the assessment of expression levels, monitoring of purification steps, and identification of impurities. The data presented highlights the sizing accuracy and robustness of the ProteoAnalyzer system across various protein expression applications.

Introduction

Ensuring the production of high-quality protein molecules is crucial for numerous industries. The production of a recombinant protein of interest involves the transcription and translation of a target DNA into an expressed protein. Recombinant protein expression can be accomplished through various techniques, including bacterial, yeast, mammalian, and insect cell fermentation, as well as cell-free in vitro expression systems. Once purified, the resulting recombinant protein is suitable for use in downstream applications including biochemical reactions, as biopharmaceuticals, or for further protein characterization studies.

The Agilent ProteoAnalyzer system is an automated quality control (QC) instrument designed to provide high accuracy and precision for quality measurements of various protein samples, including more challenging samples such as protein crude lysates.[1] The system automates traditional SDS-PAGE, eliminating extensive processing and analysis time for a more streamlined workflow.[2] Up to 12 samples can be analyzed simultaneously in approximately 30 minutes, with accurate sizing for samples ranging from 10 to 240 kDa.[3]

The ProteoAnalyzer can be used throughout the entire protein expression and purification process to assess the expression level of the desired protein, monitor purification steps, and identify any impurities present in the sample. This application note provides an example of using the ProteoAnalyzer throughout the workflow with a cell-free production system for synthesis of the calmodulin-like 3 (CALML3) protein as a representative expression system. The results illustrate the sizing accuracy and overall robustness of the ProteoAnalyzer, making it suitable for many protein expression applications.

Experimental

The Expressway Cell-Free *E. coli* Expression System (Invitrogen, p/n K9901-00) was used to produce a recombinant protein from pEXP5-NT/CALML3 plasmid DNA, which contains a His-Tag for purification, following the manufacturer's instructions. Briefly, the protein synthesis reaction was prepared as directed, and the mixture was incubated at 37 °C while shaking at 300 rpm for 30 minutes. A negative control was also prepared with no DNA template. Next, a feed mixture was prepared as directed, added to the samples, and incubated while shaking for another 5.5 hours. Aliquots of the synthesized protein and the negative control were analyzed on the ProteoAnalyzer system under reduced conditions using the LM-only method.[4] The results were compared to verify the expression of the protein of interest.

After verifying expression, the samples were precipitated with acetone, as recommended in the manual, to prevent smearing during SDS-PAGE gel analysis. SDS-PAGE gels were then compared to the ProteoAnalyzer runs.[2]

The samples were purified using the ProBond Purification System (Life Technologies, p/n K0850-01) according to the guidelines in the Expressway Cell-Free *E. coli* Expression kit manual. The supernatant from the binding step (referred to as flow-through), two wash steps, and the final elution were retained for subsequent analysis. Following purification, the purified protein and each of the supernatants were assessed under reduced conditions using the ProteoAnalyzer and with SDS-PAGE for comparison.

Results

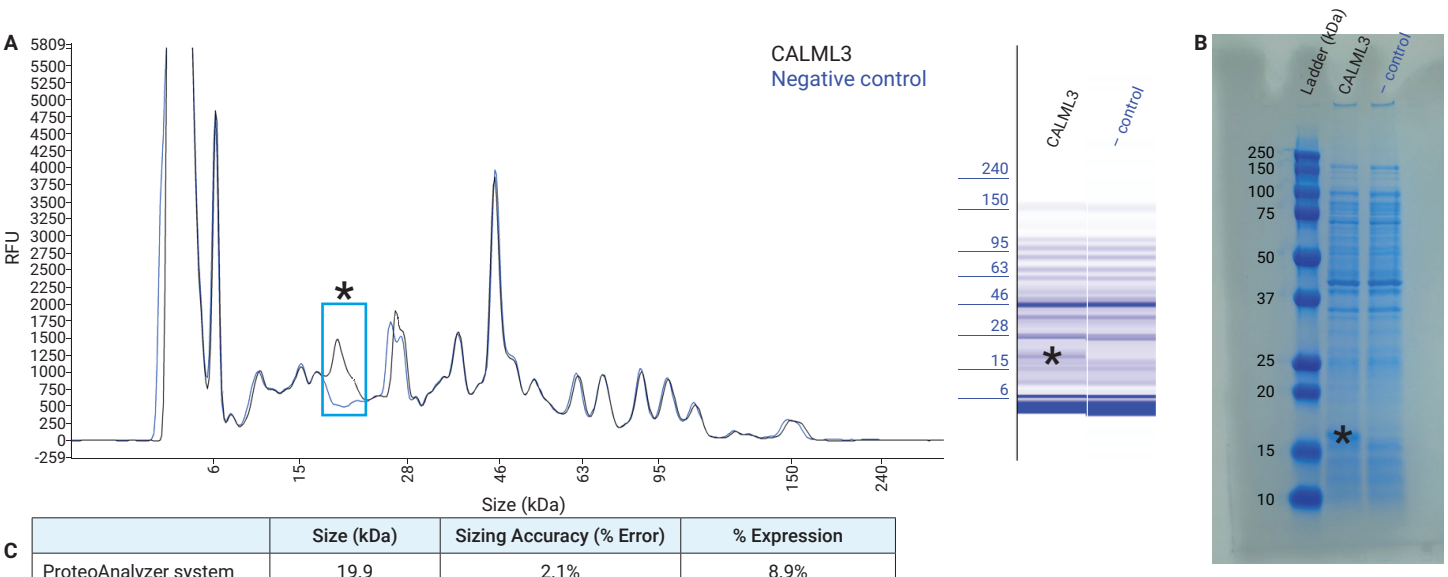
Verification of protein expression

The ProteoAnalyzer system can analyze the expression levels of specific proteins of interest. In this example, a cell-free protein expression system was used to synthesize CALML3. After electrophoretic separation, the results from the ProteoAnalyzer are automatically processed and visually represented by both an electronic gel image and an electropherogram for each sample. Additionally, a results table is generated, providing information on the size and percent expression of each sample peak identified.

Figure 1A shows an electropherogram overlay and gel view of the CALML3 protein expression reaction and the negative control. The samples were also analyzed on an SDS-PAGE gel for comparison (Figure 1B). Acetone precipitation was performed before SDS-PAGE analysis to reduce background smearing, as recommended by the kit manufacturer. This step was not necessary for analysis on the ProteoAnalyzer, resulting in faster time to results and a simpler workflow.

The CALML3 protein has an expected size of 20 kDa. Examination of this area in the electropherogram overlay demonstrates the ability of the ProteoAnalyzer to verify expression of the target protein. The ProteoAnalyzer fully resolved a peak at 19.9 kDa, which is absent in the negative control. All other proteins overlaid precisely, indicating that they were background proteins from the kit. SDS-PAGE analysis of the sample also confirmed protein expression with the presence of a band at 16 kDa. The ProteoAnalyzer displayed much better sizing accuracy, with only a 2.1% error compared to the SDS-PAGE results, which had a 17.9% error. Percent expression was assessed on the ProteoAnalyzer using a smear analysis function to identify the percentage of the total sample within the specified region. Given the expected size of the CALML3 protein, a smear range of 18 to 21 kDa was applied to assess the sample peak.

Within this range, the ProteoAnalyzer reported that the expressed CALML3 protein represented 8.9% of the total protein present in the reaction. The density of the band on the SDS-PAGE gel was also assessed, showing similar results to the ProteoAnalyzer, with 10% expression.



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	Size (kDa)	Sizing Accuracy (% Error)	% Expression
ProteoAnalyzer system	19.9	2.1%	8.9%
SDS-PAGE	16	17.9%	10.0%

Figure 1. Verification of protein expression. (A) Electropherogram overlay and digital gel image of CALML3 protein expression assessed by the Agilent ProteoAnalyzer system. (B) SDS-PAGE results. (C) Comparision of size and percent expression. Percent expression for the ProteoAnalyzer determined by the % total within a smear range of 18 to 21 kDa. * indicates CALML3.

Protein purification workflow

Following expression of a target protein, the next step is often to purify the protein for use in downstream applications. QC steps throughout this process can help to ensure a high-quality final product and assist users troubleshoot and optimize purification protocols. As described previously, the ProteoAnalyzer system is well-suited for the analysis of samples ranging from crude lysates to purified neat samples, despite the high salt buffers often used in protocols.

To demonstrate the system's capabilities, the expressed CALML3 protein was purified, and aliquots of the supernatants from each step were analyzed with the ProteoAnalyzer. Figure 2A shows the CALML3 expression profile before purification and the resulting flow-through. The sample overlay feature in the Agilent ProSize data analysis software quickly confirmed the absence of the 20 kDa peak in the flow-through, indicating that the target protein was successfully bound to the resin.

In Figure 2B, the supernatant from two subsequent wash steps was collected. Only a few impurities remain compared to the flow-through, and the amount of impurities decreases with each wash. Additionally, no protein was detected at 20 kDa indicating the successful retention of CALML3 by the resin. The ability of the ProteoAnalyzer to detect even low-level impurities is beneficial at these steps. This detection helps to determine the resin's efficiency in binding the protein of interest and whether further wash steps are required to eliminate any remaining contaminants.

Finally, the purified target protein was eluted and analyzed, as shown in Figure 2C. An overlay of the purified protein and the negative control shows the reappearance of a single peak at 20 kDa, the expected size of the CALML3 protein. The 20 kDa protein was not found in the negative control. Each of the QC steps is also shown in Figure 2D as a digital gel image, following the sample from the original expression, through each wash step, and the final purified protein present in the elution fraction.

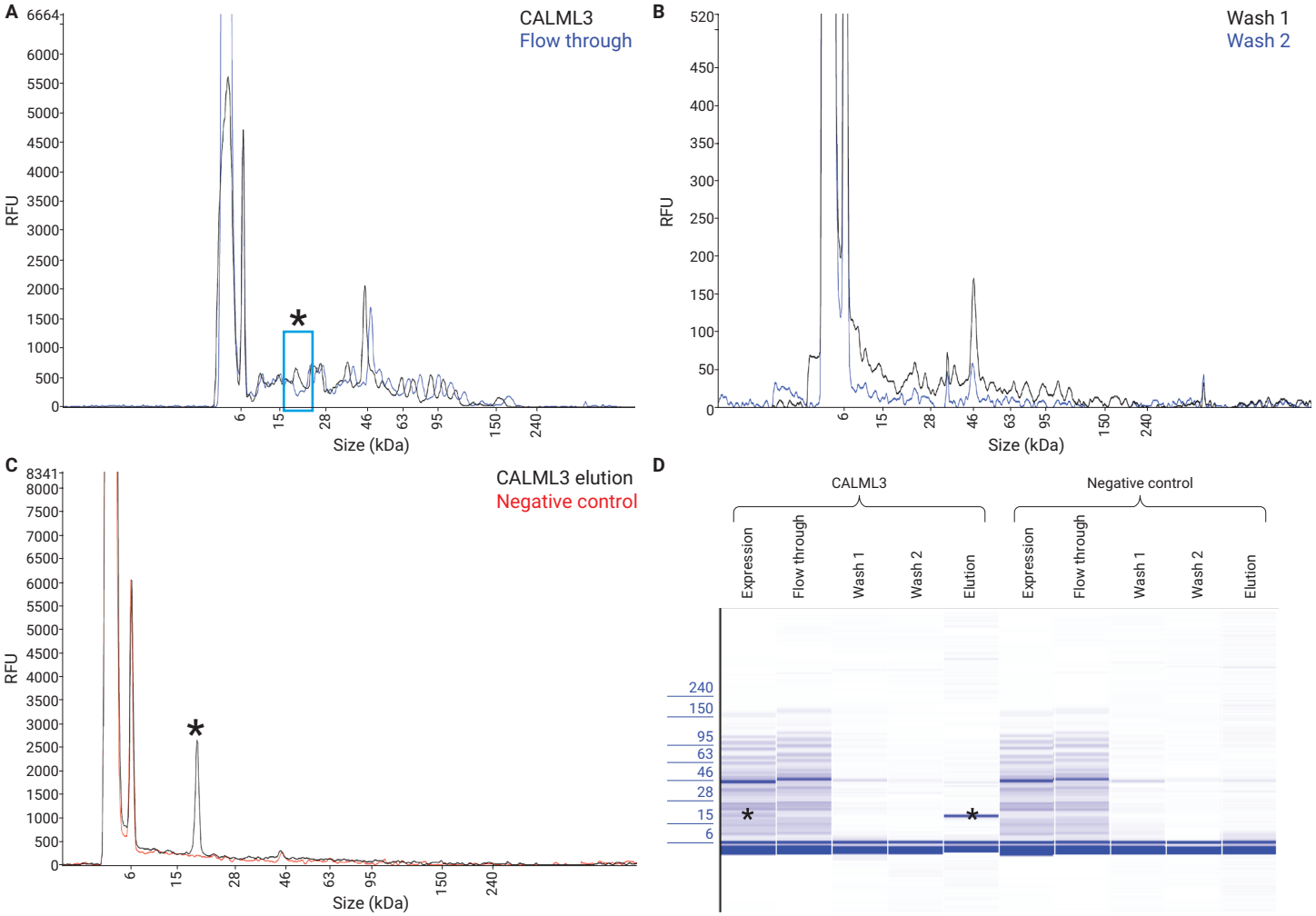

Figure 2. Verification of protein expression. (A) Electropherogram overlay and digital gel image of CALML3 protein expression assessed by the Agilent ProteoAnalyzer system. (B) SDS-PAGE results. (C) Comparision of size and percent expression. Percent expression for the ProteoAnalyzer determined by the % total within a smear range of 18 to 21 kDa. * indicates CALML3.

Sample binding, wash, and elution buffers are typically composed of high salt values. For instance, the elution buffer used in this example consists of 8M urea, 20 mM NaH_2PO_4 at pH 6.0, and 500 mM NaCl. To further illustrate the robustness of the ProteoAnalyzer when analyzing high-salt samples, the final elution was injected multiple times. The size and purity of the primary peak was determined for each injection.

Figure 3 shows an electropherogram of three subsequent injections, with the main peak displaying almost perfect overlap between injections. The purified protein displayed an average size of 19.8 kDa with excellent precision (0.3 %CV). The percent concentration, indicative of the purity of the peak, was 89.6% with a precision of 2.8 %CV. Analysis of the same sample with SDS-PAGE resulted in a size of 16 kDa and a purity of 91% (data not shown).

The sensitivity of the ProteoAnalyzer allows for the detection of incredibly low-level impurities, providing a more accurate purity assessment compared to traditional SDS-PAGE. The low %CV of both the sizing and percent purity across multiple injections highlights the reproducibility of the system. Despite the high salt concentration, the ProteoAnalyzer delivered accurate and reproducible results. Overall, the data presented throughout the expression and purification workflows demonstrates the capabilities of the ProteoAnalyzer system to analyze various protein samples.

Conclusions

Data from the protein expression and purification workflow in this application note underscore the ability of the Agilent ProteoAnalyzer system to provide precise and accurate measurements, even for challenging samples. The capability of the ProteoAnalyzer to analyze samples from crude lysates to purified proteins, with consistent detection of low-level impurities and high reproducibility, showcases the system's versatility and robustness. The Agilent ProteoAnalyzer system is well-suited for protein analysis in applications such as biotherapeutics and synthetic biology workflows.

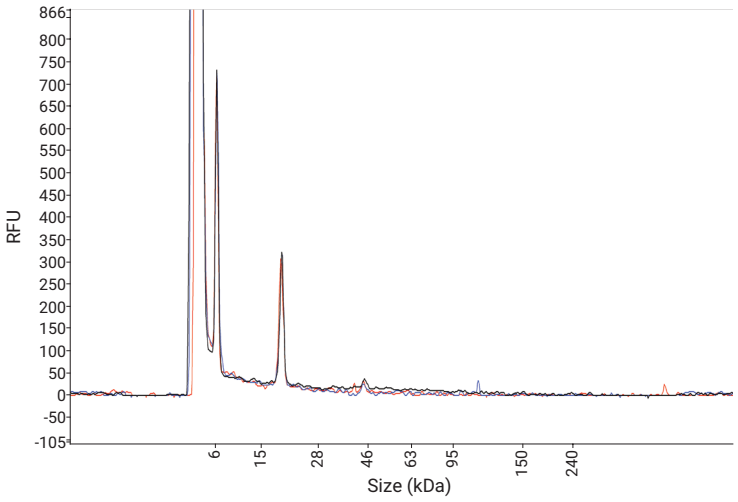

Figure 3. Overlay of three subsequent injections demonstrating the reliability and robustness of the Agilent ProteoAnalyzer system.

References

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www.agilent.com/genomics/proteoanalyzer

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