Application Note

Genomics

Agilent

Trusted Answers

Protein Purity Assessment Across the Broad Linear Dynamic Range of the Agilent ProteoAnalyzer System

Authors

Timothy Butler
Agilent Technologies, Inc.

Abstract

Reliable and accurate protein purity assessment requires the detection of both the target protein and any impurities within a linear dynamic range (LDR). If the target protein concentration exceeds the LDR while impurities remain within the LDR, purity will be undercalculated. Conversely, if the target protein is within the LDR but impurities fall below it, purity will be overcalculated. A narrow LDR can compromise the reliability of protein purity measurements. To address this, the Agilent ProteoAnalyzer system features a broad three-log LDR, enabling more robust and accurate protein purity assessments.

Introduction

Achieving accurate protein purity assessment requires detecting both the primary protein, and any impurities within the assay's LDR, as demonstrated in Figure 1A. If the target protein or impurity signals are not within the LDR, the resulting purity measurement will be under- or overcalculated. For example, in Figure 1B the target protein is within the LDR but the impurities are below the limit of detection, resulting in an overcalculation of purity. When the concentration of the target protein is greater than the limit of linearity, but the impurities are within the linear range, purity values will be undercalculated as demonstrated by Figure 1C.

The ProteoAnalyzer system automates protein purity assessment using parallel capillary electrophoresis. When assessing bovine serum albumin (BSA), the system has a three-log LDR, which increases the likelihood of both the target protein and impurities being within the optimal range for protein purity assessment (Figure 1A).[1] Traditional SDS-PAGE methods that utilize staining with dyes, such as Coomassie blue, followed by densitometry for quantitation, have limited LDRs. As a result, the probability of both the protein of interest and impurities being in the optimal range (Figure 1A) during traditional SDS-PAGE analysis is low. This application note demonstrates how users can achieve the optimal protein purity assessment (Figure 1A) by using the broad LDR of the ProteoAnalyzer compared to traditional SDS-PAGE.

Experimental

Commercially available BSA (Sigma-Aldrich; part number A7906) was diluted in 1× PBS (30 mM Tris-HCl, 26 mM NaH_2PO_4, 41 mM Na_2HPO_4, 79 mM NaCl, pH 8.5) to approximately 2,000 ng/µL, and the concentration was verified using UV absorption. The BSA protein was then serially diluted two-fold down to approximately 3.9 ng/µL in 1× PBS.

The serially diluted samples were analyzed with SDS-PAGE using precast gels (Bio-Rad; p/n 4569036) under reduced conditions. Each sample was diluted 3:1 with 4 x Laemmli buffer (Bio-Rad; p/n 161-0747), with a final concentration of 50 mM DTT. The sample was heat denatured at 90 °C for 5 minutes, then 10 µL of each concentration was loaded onto the SDS-PAGE gel. Next, 10 µL of Bio-Rad Precision Plus Protein Dual Color Standards (p/n 161-0374) was added to the wells flanking the sample lanes. Separation was conducted at 200 V for approximately 40 minutes. The gels were fixed (10% acetic acid, 40% ethanol, 50% water) for 15 minutes with rocking, then rinsed with water. The gel was stained overnight in Bio-Rad QC Colloidal Coomassie stain (p/n 1610803) and destained with deionized (DI) water for 3 hours. The experiment was repeated three times. Analysis was performed using GelAnalyzer software.[2]

Each dilution was also analyzed in triplicate under reduced conditions on the ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit (p/n 5191-6640), using the method for the optional addition of the upper marker. The system electrokinetically injected the sample at 7 kV for 10 seconds and separated the sample at 9 kV for 20 minutes. The dilutions were assessed using the Agilent ProSize data analysis software.

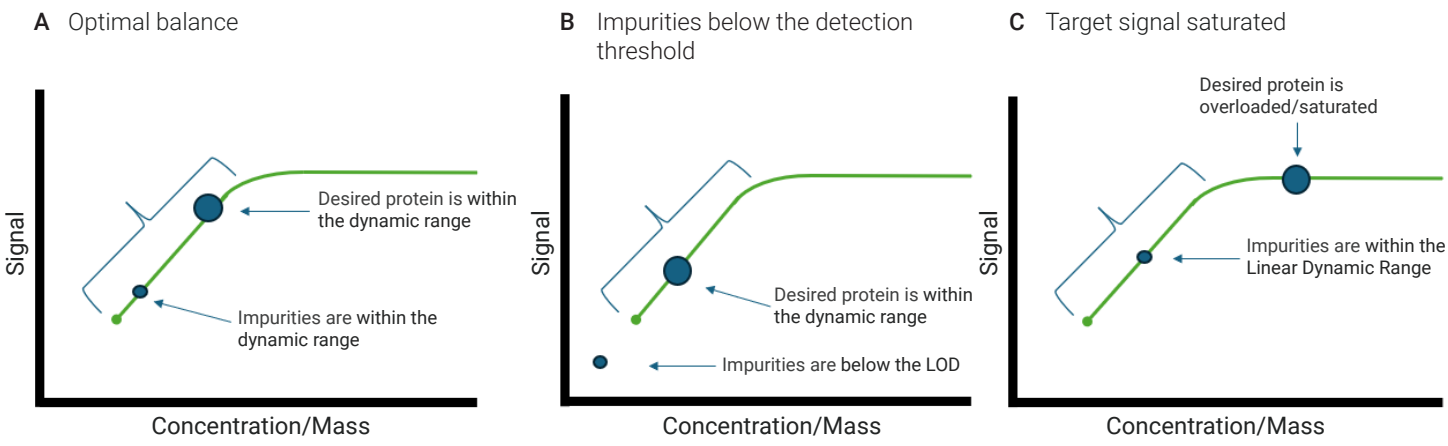

Figure 1. This illustration shows how signal levels relative to the linear dynamic range influence protein purity assessment. Accurate purity values can only be obtained when **A**) both the target protein and impurities are measured within the linear dynamic range. **B**) When the target protein signal is within the linear range but impurities are below the detection limit, purity is over calculated. Conversely, **C**) when the target protein is overloaded but impurities remain within the linear range, purity is under calculated.

Results and discussion

ProteoAnalyzer analysis

To demonstrate how the ProteoAnalyzer assesses protein purity, serially diluted BSA samples ranging from 2,044 to 3.91 ng/µL were assessed using the ProteoAnalyzer system with the Protein Broad Range P240 kit. The resulting electropherogram overlay and digital gel image show that as the concentration of the loaded samples decreased, the relative height or intensity of the peaks was reduced (Figure 2A and 2B). The target protein peaks were detectable across the full concentration range tested. However, the impurity peaks became less visible with decreasing concentrations and were undetectable at the lowest concentration, as shown by the decreasing measured peak heights of the primary impurity peak plotted in Figure 3A.

The ProteoAnalyzer has an LDR of three logs when analyzing BSA.[3] Samples in a 2.4-log span (2,044 to 7.8 ng/µL; Figure 3B) yielded consistent purity values, on average 88%, and aligned with the conditions shown in Figure 1A for optimal purity assessment. In contrast, the lowest concentration sample at 3.91 ng/µL had an average purity of 100% (Figure 3B) due to the impurity peaks falling below the LDR, consistent with the scenario illustrated in Figure 1B. The results confirm the ability of the ProteoAnalyzer to maintain a broad LDR while providing consistent purity assessment, enabling reliable performance for diverse analytical needs.

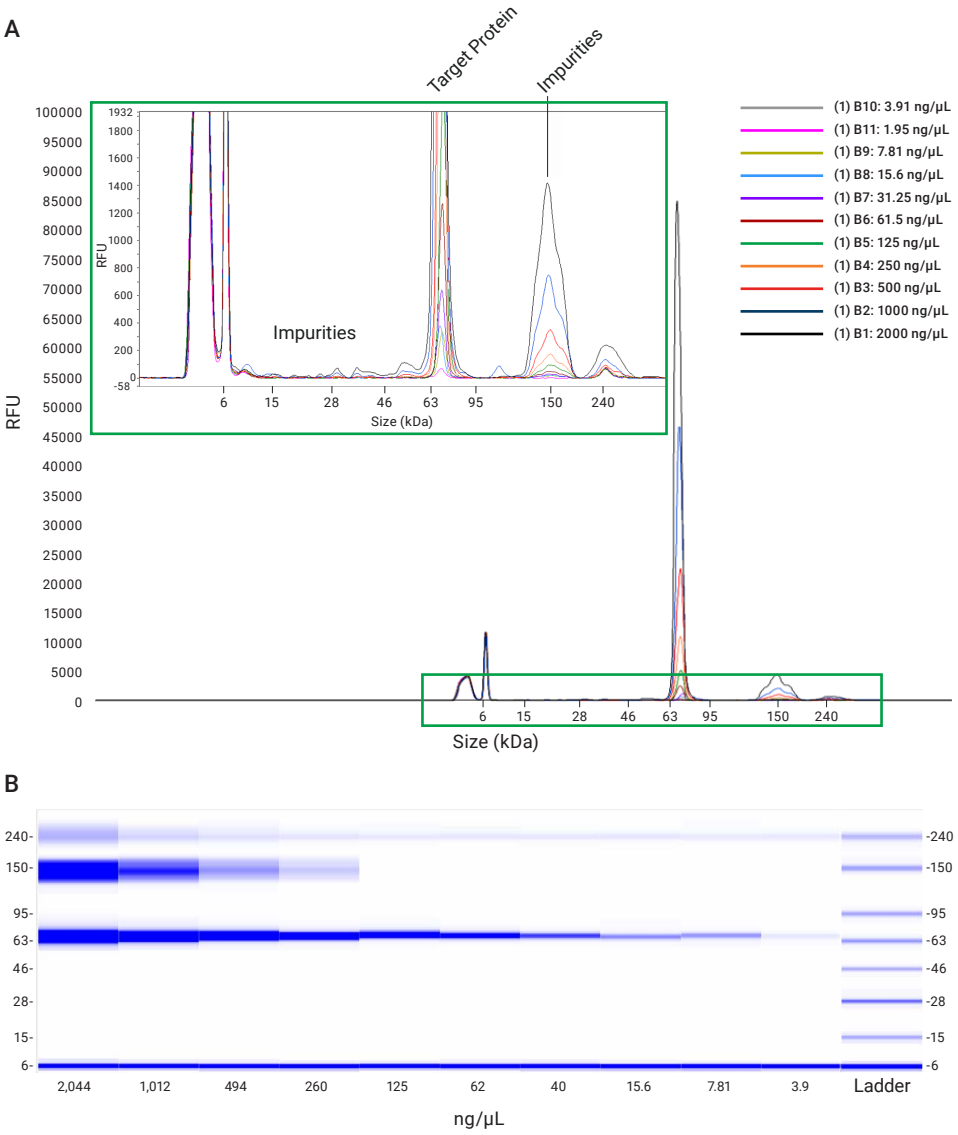

Figure 2. The BSA dilution series analyzed of the Agilent ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit shows consistent BSA detection across three logs, as illustrated by the **A**) electropherogram and **B**) digital gel image.

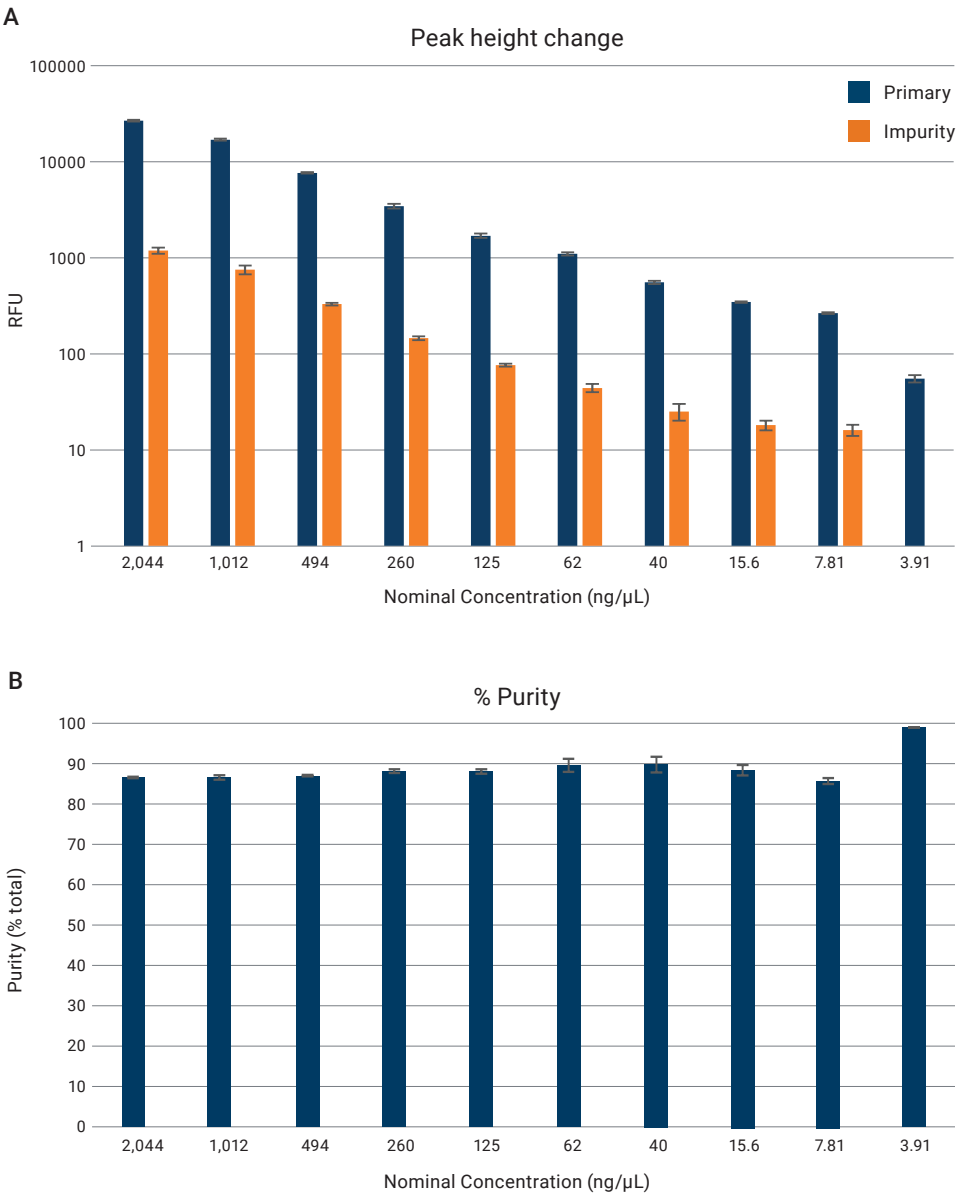

Figure 3. The signal intensity of the BSA dilution series measured on the Agilent ProteoAnalyzer system decreased as the concentration decreased, which is shown by the **A**) bar plot showing decreasing peak heights for the primary protein and the main impurity peaks. **B**) The purity bar graph demonstrates consistent purity values where both the target protein and impurities are detected for the 2,044 to 7.81 ng/µL samples. N=3, error bars represent standard deviation.

SDS-PAGE as a comparative reference

The same BSA dilution series analyzed on the ProteoAnalyzer was analyzed using SDS-PAGE with Coomassie blue staining. Examination of the resulting gel image in Figure 4A showed the three highest concentrations in the dilution series (2,044 to 494 ng/µL) as saturated bands with distorted morphology. As shown by Figure 4B, the densitometry plots for the three highest concentrations did not fit a linear trend in intensity. The remaining sample concentrations from 260 to 3.91 ng/µL showed intensities that decreased linearly with the sample mass shown by Figure 4B.

SDS-PAGE with Coomassie blue staining has an LDR of approximately two logs when assessing BSA.[3] Within the two-log range, samples from 2,044 to 494 ng/µL exhibited low purity values, ranging from 35% to 56% (Figure 4C). While the impurity peaks were detected within the linear range, the target protein was above the limit of linearity, causing the percent purity of the sample to be undercalculated, consistent with the scenario depicted in Figure 1C. At lower concentrations, from 15.6 to 3.91 ng/µL, the measured purity values were 100% (Figure 4C). The high purity resulted from impurity signals falling below the limit of detection while the target protein was within the linear range, thus resulting in an overcalculation of the purity, consistent with the conditions outlined in Figure 1B.

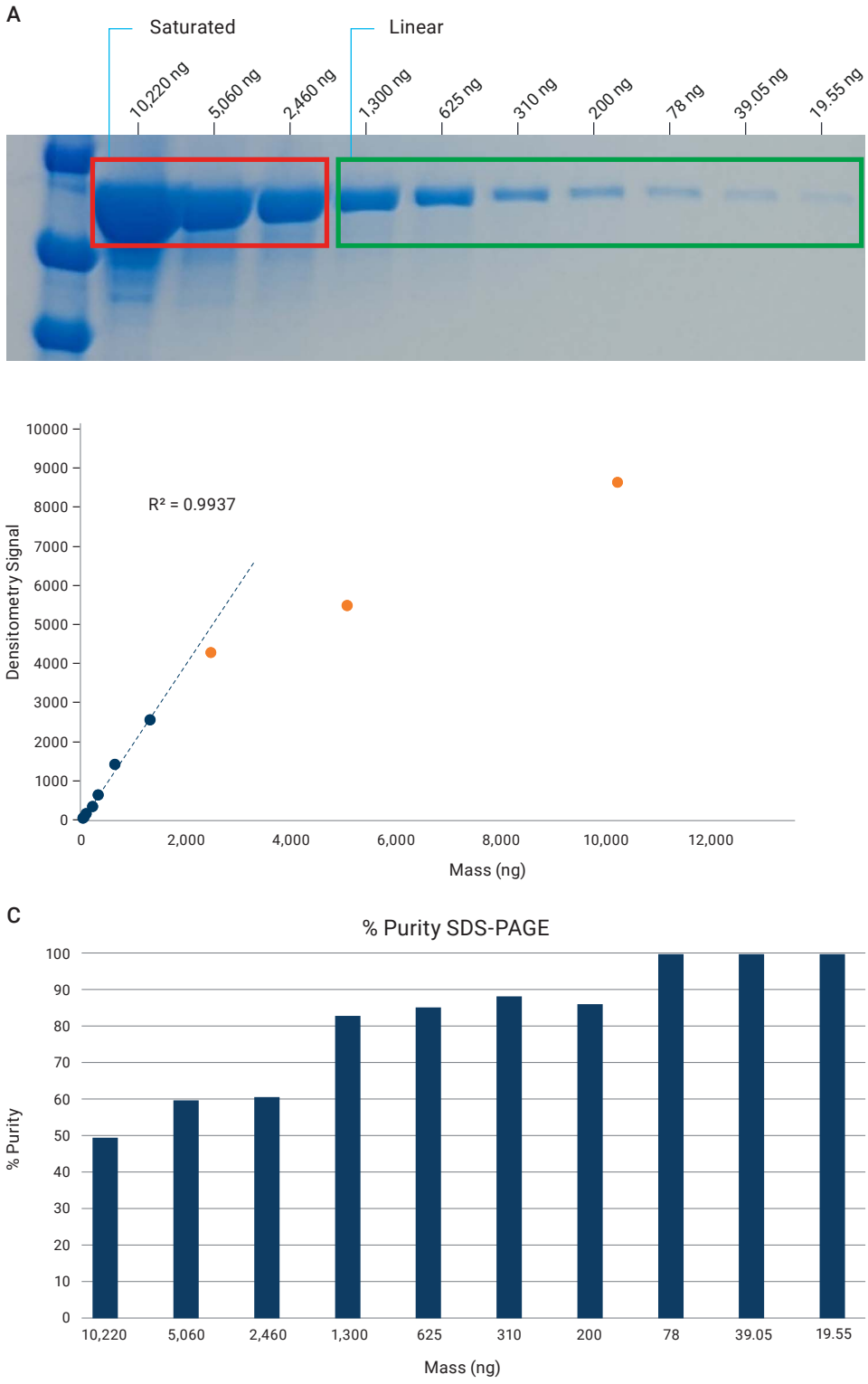

Figure 4. A) Assessment of the serially diluted BSA samples using SDS-PAGE with Coomassie Blue staining are shown by a gel image. **B**) A plot of densitometry quantitation for each sample in the serial dilution. The three highest concentrations (orange) are overloaded and non-linear (not included in linearity measurement), while the rest of the samples (blue) show linearity that continues to decrease in intensity with the lower concentration samples. **C**) The purity bar graph highlights variability in measured purity across the dilution series, emphasizing the challenge of maintaining both target and impurity signals within the limited linear dynamic range. N=1

Consistent purity measurements where both the protein of interest and impurities were in the optimal range (Figure 1A) were found for samples between 260 and 40 ng/µL, covering a range of 0.9 logs (Figure 4A, B). The purity measurements in this optimal range were between 83 and 86%, which was similar to the value reported by the ProteoAnalyzer. These results demonstrate that BSA purity measurements using densitometry detection by Coomassie Blue stain on SDS-PAGE had a narrower optimal concentration range compared to the ProteoAnalyzer for purity assessment.

Conclusion

The Agilent ProteoAnalyzer system enables robust protein purity assessments across varied concentrations by maintaining signal linearity over a three-log linear dynamic range (LDR). In this example, BSA assessed on the ProteoAnalyzer displayed an optimal LDR for reliable purity assessment of 2.4 logs, while SDS-PAGE with Coomassie Blue staining had an optimal purity assessment range of less than one log. This comparison highlights the limitations of traditional SDS-PAGE methods and demonstrates the robustness of the ProteoAnalyzer system to deliver consistent and reliable purity assessments across a wider concentration range.

Collectively, these findings highlight the importance of size of the LDR and how it affects purity assessment. While this application note used BSA as an example, other protein species may have different optimal concentration ranges for accurate purity assessment and should be determined by the user. With its broad LDR, the ProteoAnalyzer system empowers users to achieve accurate and consistent purity assessment across diverse sample concentrations beyond the capabilities of traditional approaches.

References

1. Best Practices for Protein Analysis with the Agilent ProteoAnalyzer System. *Agilent Technologies technical overview*, publication number 5994-7451, **2024**.

2. Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc. GelAnalyzer 23.1. Gelanalyzer.com. http://www.gelanalyzer.com/index.html (accessed 2025-01-07).

3. Quality Analysis Using the Agilent ProteoAnalyzer System and SDS-PAGE A; Comparison of Sizing and Quantification Performance. *Agilent Technologies technical overview*, publication number 5994-6934EN, **2023**.

www.agilent.com/genomics/proteoanalyzer

PR7004-935

Published in the USA, January 14, 2026
5994-8818EN

Agilent
Trusted Answers