Technical Overview

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Analysis of Protein Size and Concentration using the Agilent ProteoAnalyzer and Agilent 2100 Bioanalyzer Systems

Introduction

Accurate and precise assessment of protein size and concentration is a necessary component of quality control (QC) procedures. Such measurements are needed to verify the success of protein extraction and purification processes. To obtain reliable QC outcomes, choice of instrument for protein sample analysis is a critical factor.

The Agilent ProteoAnalyzer system is a capillary electrophoresis sodium dodecyl sulfate (CE-SDS) instrument designed to facilitate precise and accurate measurements of up to 12 protein samples in a run, and up to 96 total samples, without repeated user interaction. A lower marker (LM) of known size and concentration is added to each sample for alignment and quantitation. An optional upper marker (UM) can be added for improved alignment and more accurate sizing.[1]

The Agilent 2100 Bioanalyzer system achieves separation using microfluidic-chip-based technology. When analyzing proteins, the system is compatible with two standard sensitivity kits that both use an LM and UM for accurate and precise sizing and quantification. In addition, the Bioanalyzer has a high sensitivity kit that only uses an LM. The system can analyze up to 10 samples at a time before needing further user action.

In this technical overview, the Agilent Protein Broad Range P240 kit used with the ProteoAnalyzer and the Agilent Protein 230 assay used with the Bioanalyzer were compared. The sizing accuracy and precision, and the quantification reproducibility of two model protein samples, Bovine Serum Albumin (BSA) and Carbonic Anhydrase II (CAII) from Bovine, was demonstrated on both instruments and compared to the listed kit specifications (Table 1).

Experimental

Commercially available BSA (Sigma, part number A7906) and CAII proteins (Sigma, part number C2273-1VL) were prepared at a concentration of 2,000 ng/µL in 1X PBS and verified using UV absorption at 280 nm. Each protein was then serially diluted two-fold to an ending expected concentration of 3.9 ng/µL. Each concentration of the samples was analyzed in triplicate under reduced conditions on the Agilent ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit (part number 5191-6640)[2] using the optional upper marker method. Samples were also analyzed using the Agilent 2100 Bioanalyzer system under reduced conditions using the Agilent Protein 230 assay (part number 5067-1517). Sizing and concentration results from the analysis of BSA and CAII were compared between each system.

Table 1. Kit specifications of the Agilent Protein Broad Range P240 kit used with the Agilent ProteoAnalyzer system, and the Agilent Protein 240 assay used with the Agilent 2100 Bioanalyzer system.

	ProteoAnalyzer System		2100 Bioanalyzer System
Analytical specifications	**Method**	**Protein Broad Range P240 kit**	**Protein 230 kit**
Sizing Range	LM only	10 to 240 kDa	14 to 230 kDa
	LM and UM	10 to 200 kDa	
Typical Sizing Accuracy (% Sizing Error)	LM only	< 15% for BSA, CAII (using reduced conditions)	N/A
	LM and UM	< 10% for BSA, CAII (using reduced conditions)	10% for BSA, CAII
Typical Resolution		< 10% molecular weight resolution between 15 to 150 kDa (based on ladder) R ≥ 1 NIST mAb NGHC/HC (using reduced conditions)	10%
Sizing Precision	LM only	< 8 %CV for BSA, CAII, GREMLIN-1 and NIST mAb (using reduced conditions) < 10 %CV for intact NIST mAb (using non-reduced conditions)	N/A
	LM and UM	< 5 %CV for BSA, CAII, GREMLIN-1 and NIST mAb (using reduced conditions)	3 %CV BSA, CAII
Quantitative Range		2 ng/µL to 2,000 ng/µL for BSA in PBS	15 to 2,000 ng/µL for CAII in PBS 30 to 2,000 ng/µL for BSA in PBS
Sensitivity (Signal/Noise > 3)		1 ng/µL for BSA, CAII in PBS	6 ng/µL for CAII in PBS 15 ng/µL for BSA in PBS 30 ng/µL for BSA in 0.5 M NaCl
Quantification Reproducibility		<15 %CV for 20 – 2,000 ng/µL <25 %CV for 2 – 20 ng/µL BSA	20 %CV (BSA, CAII)

Results

Visual analysis

To detect samples on either the ProteoAnalyzer or Bioanalyzer, dyes are used that fluoresce when exposed to the correct wavelength of light. The ProteoAnalyzer uses a covalent dye for detection of proteins, while the Bioanalyzer uses an associative dye. Despite this difference, the systems can both be used for reliable sizing and concentration analysis of proteins. With either instrument, samples can be visualized as either a digital gel image of all samples or individual electropherograms of each sample. Figure 1 shows representative electropherograms of BSA and CAII from both systems. Both the ProteoAnalyzer and Bioanalyzer display the sample as a peak flanked on either side by an LM and UM to allow for alignment and sizing of the sample. A system peak from the dye is present with both analysis methods, but in different locations. The system peak on the ProteoAnalyzer is located before the LM, preventing any sample interference. The system peak on the Bioanalyzer is located after the LM and may interfere with analysis of smaller samples. Still, both instruments can analyze proteins with high degrees of accuracy and precision.

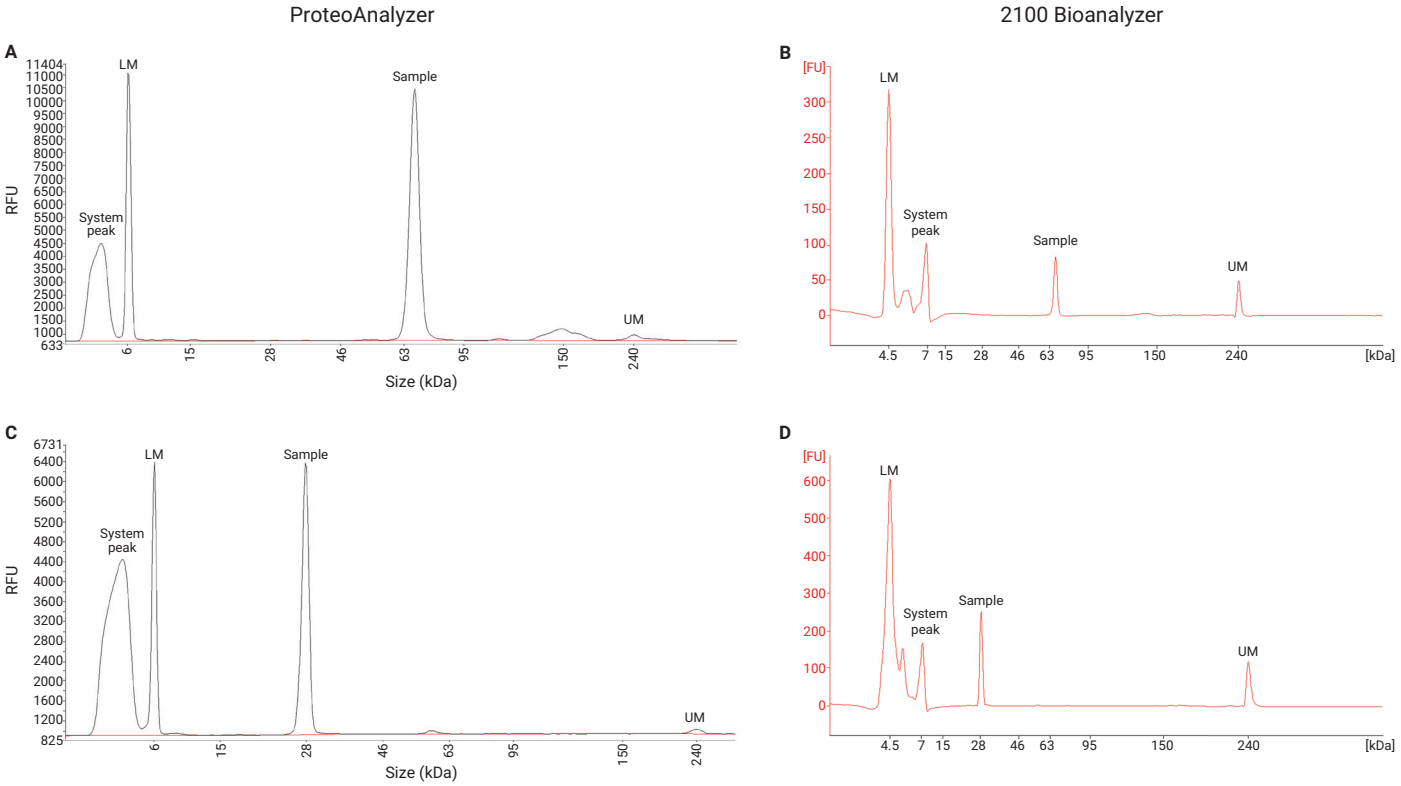

Figure 1. Protein analysis was compared on the Agilent ProteoAnalyzer system and the Agilent 2100 Bioanalyzer system. Shown are representative electropherograms of BSA at a concentration of 250 ng/µL on the A) ProteoAnalyzer and B) Bioanalyzer; and CAII at a concentration of 250 ng/µL on the C) ProteoAnalyzer and D) Bioanalyzer.

Sizing accuracy and precision

The specifications for both the ProteoAnalyzer and Bioanalyzer protein analysis kits define sizing accuracy and precision based on measurements using BSA and CAII (Table 1). BSA has a known size of 66 kDa. The average size for BSA across the serial dilutions using the ProteoAnalyzer and Bioanalyzer were 69.2 and 67.1 kDa, respectively. Figure 2A shows each instrument's average size measurements across the dilution range for BSA. When compared to the known size of BSA, the ProteoAnalyzer showed an overall average sizing error of 4.81%, while the Bioanalyzer reported a sizing error of 1.90%. In addition, the overall average precision across the serial dilutions when measuring BSA on the ProteoAnalyzer, represented by the coefficient of variation (%CV), was 0.66%. On the Bioanalyzer, this value was 0.51% (Figure 2B). The data presented here, and further assessment of each dilution, indicates that measurements of BSA on both instruments were highly accurate and precise.

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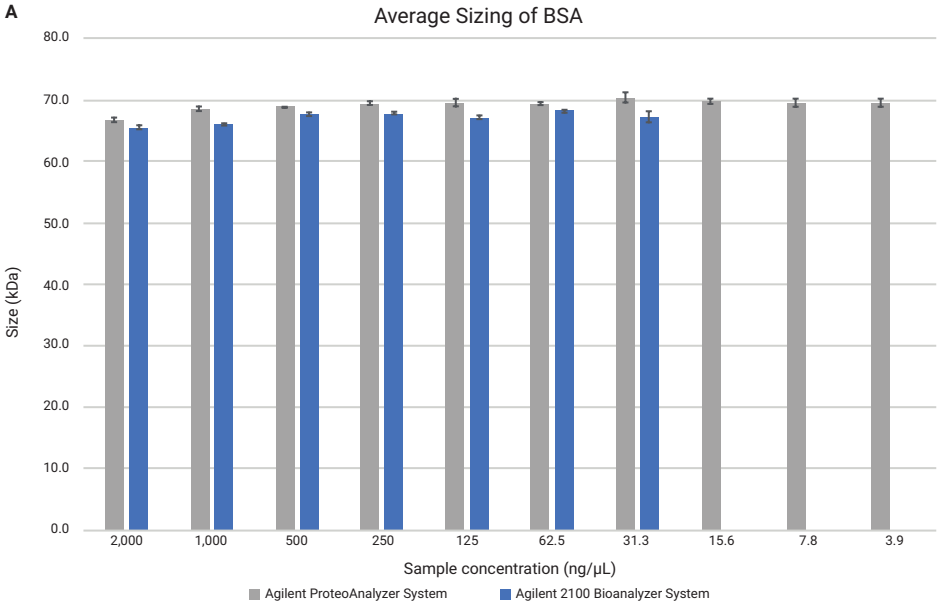

B

BSA Sizing Analysis						
	Average Size (kDa)		Accuracy (%Error)		Precision (%CV)	
Nominal Concentration	ProteoAnalyzer	2100 Bioanalyzer	ProteoAnalyzer	2100 Bioanalyzer	ProteoAnalyzer	2100 Bioanalyzer
2,000 ng/µL	66.7	65.5	1.01%	–0.71%	0.57%	0.49%
1,000 ng/µL	68.5	66.0	3.84%	–0.05%	0.59%	0.23%
500 ng/µL	69.0	67.6	4.49%	2.47%	0.08%	0.23%
250 ng/µL	69.4	67.8	5.10%	2.78%	0.46%	0.45%
125 ng/µL	69.6	67.1	5.40%	1.67%	0.87%	0.54%
62.5 ng/µL	69.4	68.2	5.10%	3.28%	0.46%	0.37%
31.3 ng/µL	70.4	67.3	6.62%	1.92%	1.37%	1.26%
15.6 ng/µL	69.8	*	5.71%	*	0.54%	*
7.8 ng/µL	69.6	*	5.40%	*	0.79%	*
3.9 ng/µL	69.6	*	5.40%	*	0.87%	*
Overall Average	**69.2**	**67.1**	**4.81%**	**1.90%**	**0.66%**	**0.51%**

**Outside kit range*

Figure 2. Average reported sizes for two-fold serially diluted BSA with a concentration range of 2,000 to 3.9 ng/µL using either the Agilent ProteoAnalyzer system with the Protein Broad Range P240 kit, or the Agilent 2100 Bioanalyzer system with the Protein 230 assay. A) Average reported size across the concentration range of the kits, with error bars representing standard deviation. B) Table showing average size, % error, and %CV for each BSA dilution (n=3).

For CAII with a known size of 29 kDa, the average size across the serial dilutions reported by the ProteoAnalyzer and Bioanalyzer were 28.0 kDa and 28.9 kDa, respectively. Figure 3A shows the observed average sizes of each dilution measured by the ProteoAnalyzer and Bioanalyzer. The overall accuracy of the ProteoAnalyzer, represented by the average percent error, was 3.55% across the dilution range, while the Bioanalyzer demonstrated a percent error of 0.31%. The %CV for CAII on the ProteoAnalyzer was 1.04% compared to 1.87% when using the Bioanalyzer (Figure 3B). Taken together with the evaluation of each dilution, the data demonstrates that measurements of CAII were characterized by exceptional accuracy and precision on both instruments.

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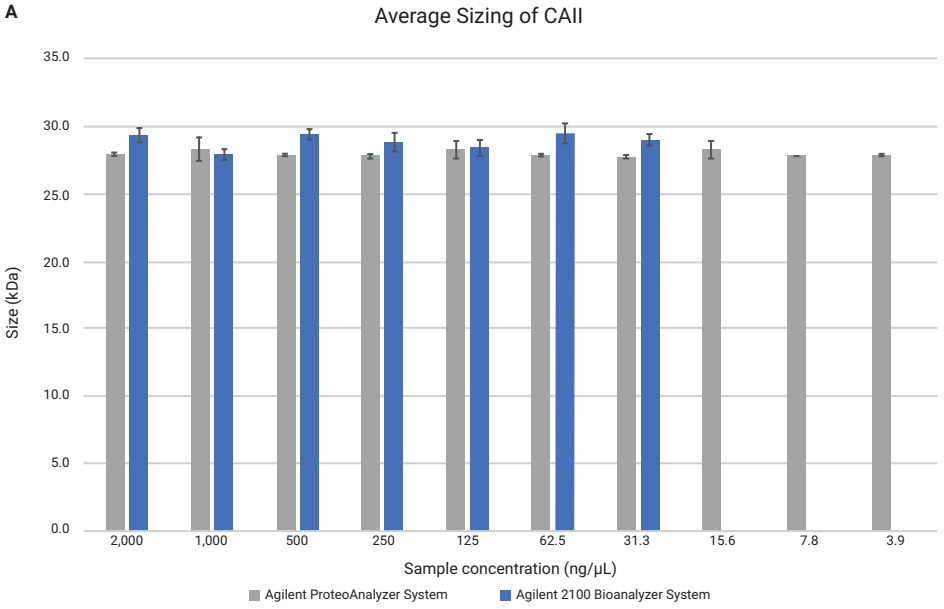

B

CAII Sizing Analysis						
	Average Size (kDa)		Accuracy (%Error)		Precision (%CV)	
Nominal Concentration	ProteoAnalyzer	2100 Bioanalyzer	ProteoAnalyzer	2100 Bioanalyzer	ProteoAnalyzer	2100 Bioanalyzer
2,000 ng/µL	27.9	29.3	−3.68%	1.15%	0.41%	1.72%
1,000 ng/µL	28.3	27.9	−2.41%	−3.68%	3.06%	1.45%
500 ng/µL	27.9	29.4	−3.91%	1.38%	0.41%	1.23%
250 ng/µL	27.8	28.8	−4.14%	−0.69%	0.72%	2.43%
125 ng/µL	28.3	28.4	−2.53%	−1.95%	2.27%	2.15%
62.5 ng/µL	27.9	29.5	−3.91%	1.61%	0.41%	2.50%
31.3 ng/µL	27.7	29.0	−4.37%	0.00%	0.42%	1.58%
15.6 ng/µL	28.3	*	−2.53%	*	2.27%	*
7.8 ng/µL	27.8	*	−4.14%	*	0.00%	*
3.9 ng/µL	27.9	*	−3.91%	*	0.41%	*
Overall Average	**28.0**	**28.9**	**−3.55%**	**−0.31%**	**1.04%**	**1.87%**

**Outside kit range*

Figure 3. Average reported sizes for two-fold serially diluted CAII with a concentration range of 2,000 to 3.9 ng/µL using either the Agilent ProteoAnalyzer system with the Protein Broad Range P240 kit, or the Agilent 2100 Bioanalyzer system with the Protein 230 assay. A) Average size of CAII samples across the concentration range of the kits. B) Table showing average size, % error, and %CV for each CAII dilution (n=3).

Quantification

To compare the quantification capabilities of the ProteoAnalyzer and Bioanalyzer, Figure 4 illustrates the correlation between the measured concentrations and the nominal concentrations of BSA and CAII on each system. Linearity was calculated for each system, as indicated by the R^2 values. The ProteoAnalyzer exhibited an R^2 of 0.9995 for BSA (Figure 4A) and 0.9993 for CAII (Figure 4B), indicating a strong linear relationship between the sample concentrations and the instrument measurements. Similarly, the Bioanalyzer demonstrated an R^2 of 0.9968 for BSA (Figure 4A) and 0.9478 for CAII (Figure 4B), signifying high degrees of linearity.

The precision of each instruments' quantification measurements is demonstrated by calculating the %CV from analysis of multiple replicates of each serially diluted sample (Figure 4C). BSA samples on the ProteoAnalyzer displayed an average of 2.58 %CV, while the Bioanalyzer showed 7.51 %CV. For CAII run on the ProteoAnalyzer, an average of 5.92 %CV was calculated, while the Bioanalyzer showed an average of 6.78 %CV. The ProteoAnalyzer showed an overall lower %CV than the Bioanalyzer for both proteins tested. Both systems showed %CV values within the range of their kits. The low %CVs demonstrate that each instrument provides precise results across multiple runs.

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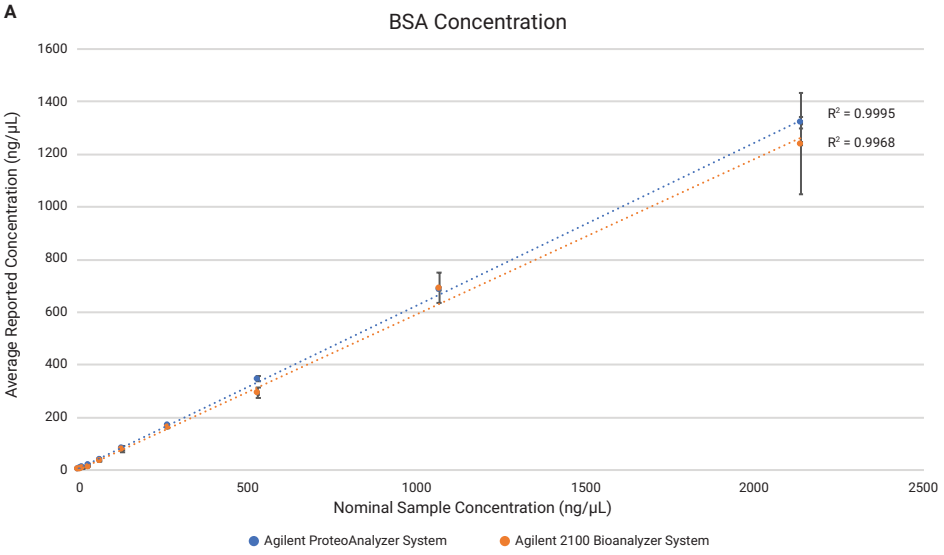

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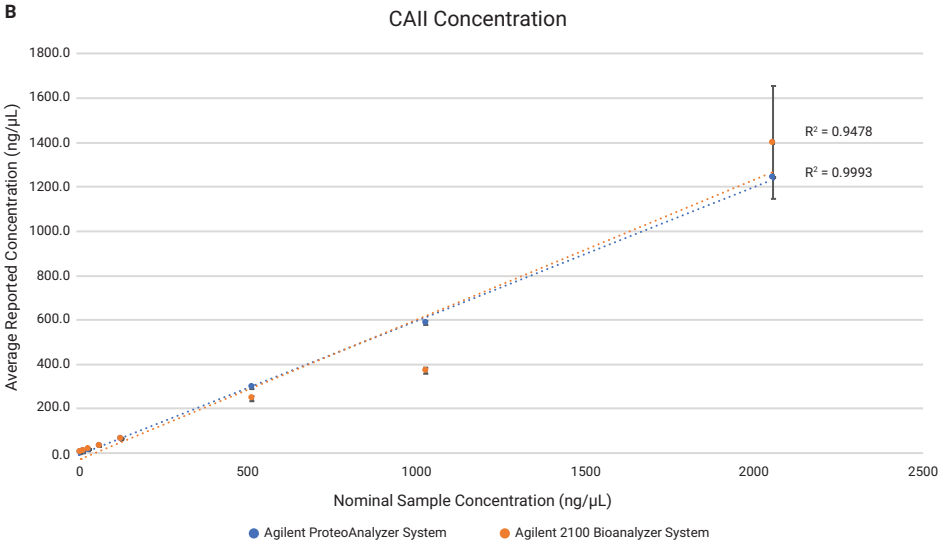

C

Quantification Precision (%CV)				
	BSA		CAII	
Nominal Concentration	ProteoAnalyzer	2100 Bioanalyzer	ProteoAnalyzer	2100 Bioanalyzer
2,000 ng/µL	1.28%	15.60%	7.44%	18.11%
1,000 ng/µL	1.45%	8.23%	1.34%	3.45%
500 ng/µL	0.74%	6.59%	2.38%	4.46%
250 ng/µL	1.33%	0.50%	5.91%	2.45%
125 ng/µL	1.99%	15.72%	2.37%	2.99%
62.5 ng/µL	2.23%	0.27%	4.61%	6.34%
31.3 ng/µL	1.35%	5.68%	8.96%	9.63%
15.6 ng/µL	3.09%	*	6.12%	*
7.8 ng/µL	4.46%	*	11.45%	*
3.9 ng/µL	7.84%	*	8.65%	*
Average	**2.58%**	**7.51%**	**5.92%**	**6.78%**

**Outside kit range*

Figure 4. Comparisons of linearity and precision between the Agilent ProteoAnalyzer and Agilent 2100 Bioanalyzer systems' quantification capabilities. Shown are correlations between the nominal concentration of each dilution against the observed concentrations for both A) BSA and B) CAII. C) Precision of each system when measuring BSA and CAII concentration is represented as %CV results.

Conclusion

In this technical overview, a comprehensive comparison was conducted between the Agilent ProteoAnalyzer and Agilent Bioanalyzer systems, using BSA and CAII as model proteins. Both instruments exhibited exceptional accuracy and precision in sizing protein samples and performed well within their kit specifications. Furthermore, both systems show a high level of quantification precision and high linearity between measured and nominal concentrations, assuring users of reliable results. The ProteoAnalyzer offers distinct advantages over the Bioanalyzer. These include broader kit sizing and concentration ranges, no chip handling, unobstructed detection of smaller proteins, and higher throughput for up to 96 samples. The ProteoAnalyzer can address the ever-changing needs in modern protein analysis by delivering higher throughput and reliable results.

References

1. Protein Sizing and Quantification With the Agilent 5350 ProteoAnalyzer System, *Agilent Technologies Technical Overview*, publication number 5994-6718EN, **2023**.
2. Agilent Protein Broad Range P240 Kit. *Agilent Technologies data sheet*, publication number 5994-6688EN, **2023**.

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