Application Note

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Cytokine and Growth Factor Quality Control Analysis with Automated CE-SDS

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Abstract

This application note highlights the capabilities of the Agilent ProteoAnalyzer system for size and purity analysis of various cytokines and growth factors. The ProteoAnalyzer demonstrated superior sensitivity compared to traditional SDS-PAGE for the detection of monomeric, dimeric, and trimeric forms of proteins, as well as small fragments not visible with SDS-PAGE. The system provided highly accurate and reproducible sizing measurements. Additionally, the ProteoAnalyzer assessed sample purity, reporting the percentage of each detected peak relative to the total concentration with high reproducibility. Overall, the ProteoAnalyzer system significantly enhances the quality and reliability of protein analysis.

Introduction

The Agilent ProteoAnalyzer system is a parallel capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) instrument that automates analysis of reduced and nonreduced protein samples. The system can separate up to 12 samples in about 30 minutes. With a sizing range of 10 to 240 kDa, and discrete individual capillaries that are rejuvenated between each run, the ProteoAnalyzer can be used to assess a wide variety of samples, including monoclonal antibodies, protein purification fractions, and crude lysates.[1]

Growth factors and cytokines are signaling molecules that regulate cell growth and differentiation, regulate immune and inflammatory response, and function as biomarkers for many diseases. These proteins are vital tools in cell culture systems, allowing researchers to mimic physiological and pathological conditions to study cell behavior, signal transduction, immune responses, and cellular development. Factors that can affect cytokine and growth factor levels in cell culture include the biological source, sample processing, and sensitivity to freeze-thaw[2]. In cell culture, these proteins must be of high quality to ensure consistent and reliable experimental outcomes. This application note provides examples of the analysis of growth factors and cytokines using the ProteoAnalyzer system, with particular emphasis on sizing and purity analysis.

Experimental

A variety of commercially available RUO recombinant human cytokines and growth factors were obtained from Sartorius, including IL-18 (p/n CYK-0025-1015), VEGF 165 (p/n CYK-0010-0005), TNF-alpha (p/n CYK-0025-1004), and IL-15 (CYK-0025-1003). Each sample was reconstituted at 1 µg/µL in 1x PBS per manufacturer's instructions.

The samples were prepared in 1x PBS at a concentration of 500 ng/µL under both reduced and nonreduced conditions and covalently labeled according to the Agilent Protein Broad Range P240 kit quick guide[3]. Each sample was analyzed in triplicate on the Agilent ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit (p/n 5191-6640) using the LM-only and the LM and UM methods[3]. Sizing and quantification were assessed using the Agilent ProSize data analysis software.

The samples were also analyzed with SDS-PAGE using precast gels (Bio-Rad; part number 4569036) under reduced and nonreduced conditions. One microgram of sample was mixed with Laemmli buffer (Bio-Rad; part number 161-0747), heat denatured at 90 °C for five minutes, and loaded on the gel. Analysis was performed using GelAnalyzer software[4] for sizing and quantification.

Results

Various cytokines and growth factors were analyzed on the ProteoAnalyzer system under both reduced and nonreduced conditions. The Agilent ProSize data analysis software provides the results as both an electronic gel image and an electropherogram, along with a table of results including information such as size and percent total of individual peaks.

To demonstrate the capabilities of the ProteoAnalyzer system, a variety of growth factors and cytokines were assessed for size and purity under both reduced and nonreduced conditions. Each sample was also analyzed using SDS-PAGE for comparison. The ProteoAnalyzer was able to resolve and detect the expected peaks in each sample. In comparison, SDS-PAGE gels were generally only able to detect the monomeric forms of each sample. For example, the ProteoAnalyzer was able to resolve and detect the expected monomer, dimer, and trimer peaks of IL-18, while SDS-PAGE was only able to detect the monomer (Figure 1).

The expected size of the three peaks of IL-18 is 18.2, 36.4, and 54.6 kDa. The average size of each of the peaks reported by the ProteoAnalyzer was very close to the expected, with less than 10% error for each peak. The monomeric peak showed an average size of 19.7 under reduced conditions, with excellent precision of 0.59 %CV. Under nonreduced conditions, the monomer displayed an average size of 20.0, dimer 36.0, and trimer 51.2 kDa. Each measurement displayed excellent precision of less than 2.3 %CV. In contrast, analysis with SDS-PAGE resulted in a size of 16 kDa for the monomeric sample under both reduced and nonreduced conditions, and was not able to detect the dimer or the trimer. Comparison of the IL-18 sizing between systems showed approximately an 11% difference in sizing of the monomeric peak.

The specifications for sizing accuracy and precision using the ProteoAnalyzer are based on a set of model proteins, including BSA, CAII, GREMLIN-1, and NIST mAb. The average sizing of each of the growth factors and cytokines tested in this study is summarized in Table 1. The typical sizing accuracy for model proteins is less than 15% error under reduced conditions. For the cytokine and growth factors tested in this application note, the sizing accuracy varied, with the monomeric peak under reduced conditions being between 8 and 22% error. This could be due to variations in the amino acid sequence, isoelectric point (pI), charge, hydrophobicity, or other protein characteristcs that can influence the SDS binding ratio to the protein, altering the mass-to-charge ratio and thus the apparent size of the protein during electrophoretic analysis. The sizing reported by the ProteoAnalyzer was similar to that of SDS-PAGE, with no more than a 13% difference between the systems for the samples under reduced conditions. Overall, the ProteoAnalyzer displayed excellent precision in sizing measurements, with the majority of the peaks in each sample having a %CV less than 2.7. The sizing precision for all peaks was well within the kit specifications of less than 8% CV using reduced conditions and the LM method[1].

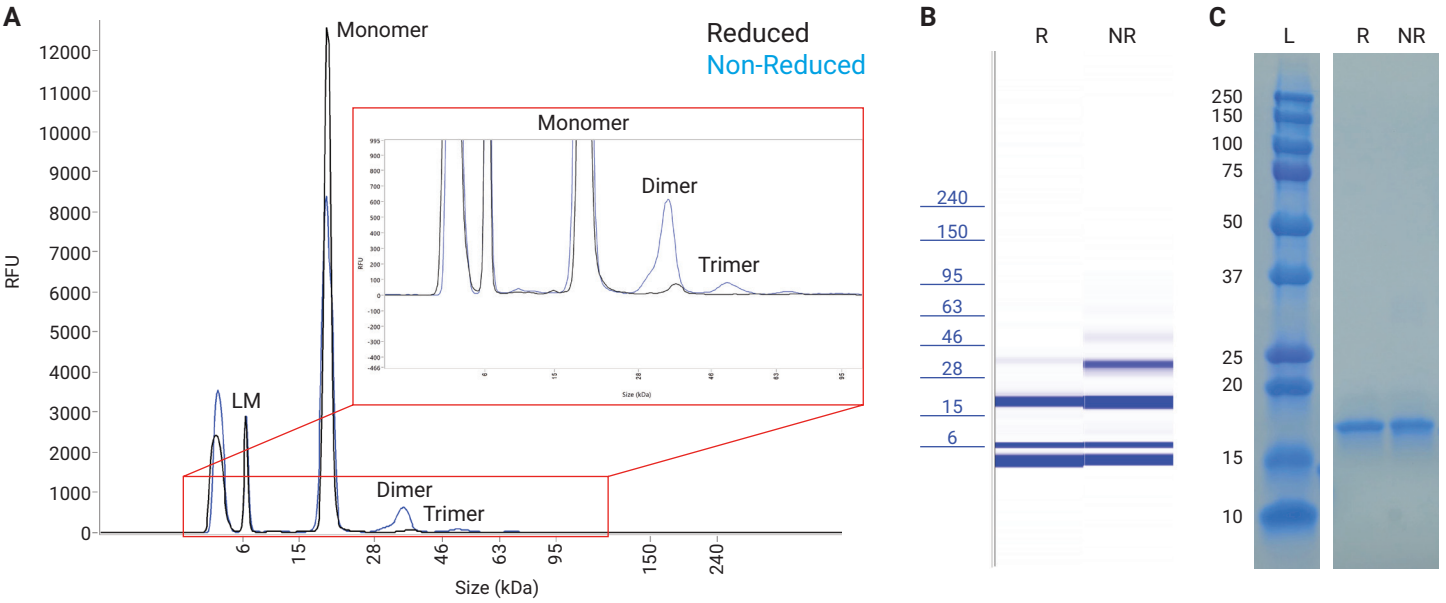

Figure 1. Analysis of IL-18 on the Agilent ProteoAnalyzer system. A) Electropherogram, B) digital gel image. C) SDS-PAGE. LM: Lower marker, L: ladder; R: Reduced; NR: Non-reduced

Table 1. Sizing analysis of growth factors and cytokines assessed on the Agilent ProteoAnalyzer system and with SDS-PAGE.

				ProteoAnalyzer			SDS-PAGE		
Protein	**Conditions**		**Theoretical Size (kDa)**	**Average Size (kDa)**	**Precision (%CV)**	**Accuracy (% error)**	**Average Size (kDa)**	**Accuracy (% error)**	**ProteoAnalyzer - SDS-PAGE % Difference**
IL-18	**Reduced**		18.2	19.7	0.59	8.4	16	12.1	10.4
	Nonreduced	**Monomer**	18.2	20.0	1.32	9.9	16	12.1	11.1
		Dimer	36.4	36.0	2.26	1.0	ND	NA	NA
		Trimer	54.6	51.2	1.98	6.3	ND	NA	NA
VEGF-165	**Reduced**		19.0	22.6	1.35	19.1	18	5.3	11.4
	Nonreduced	**Monomer**	19.0	ND	NA	NA	ND	NA	NA
		Dimer	38.0	42.0	6.25	10.6	36	5.3	7.7
		Trimer	57.0	67.3	1.34	18.0	ND	NA	NA
TNF-alpha	**Reduced**		17.5	19.4	1.07	11.1	1	8.6	9.7
	Nonreduced	**Monomer**	17.5	18.6	2.46	6.3	16	8.6	7.5
		Dimer	35.0	34.8	2.32	0.5	33	5.7	2.7
		Trimer	52.5	54.2	1.86	3.3	ND	NA	NA
		Tetramer	70.0	76.9	2.22	9.9	ND	NA	NA
		Pentamer	87.5	101.3	1.09	15.8	ND	NA	NA
IL-15	**Reduced**		13.7	16.7	1.20	21.9	13	5.1	12.5
	Nonreduced	**Monomer**	13.7	12.5	1.39	8.8	11	19.7	6.4
		Dimer	27.4	24.4	2.73	10.8	ND	NA	NA

Following analysis with the ProteoAnalyzer, ProSize also reports the percentage of each peak detected in comparison to the total concentration. The percent total is representative of the purity of the sample. In the example shown in Figure 2, TNF-alpha was analyzed under reduced and nonreduced conditions on the ProteoAnalyzer. Under reduced conditions, the monomeric peak represents 96.6% of the total concentration of the sample loaded. The high sensitivity of the ProteoAnalyzer allowed for detection and purity assessment of several multimers under non-reduced conditions, with the monomeric peak having a purity of 75.3%, the dimer 17.4%, and the trimer, tetramer, and pentameter each approximately 4% or less. Additionally, the system was highly reproducible, with precision values of 1.1%CV or less for the reduced form of each of the five proteins tested (Table 2).

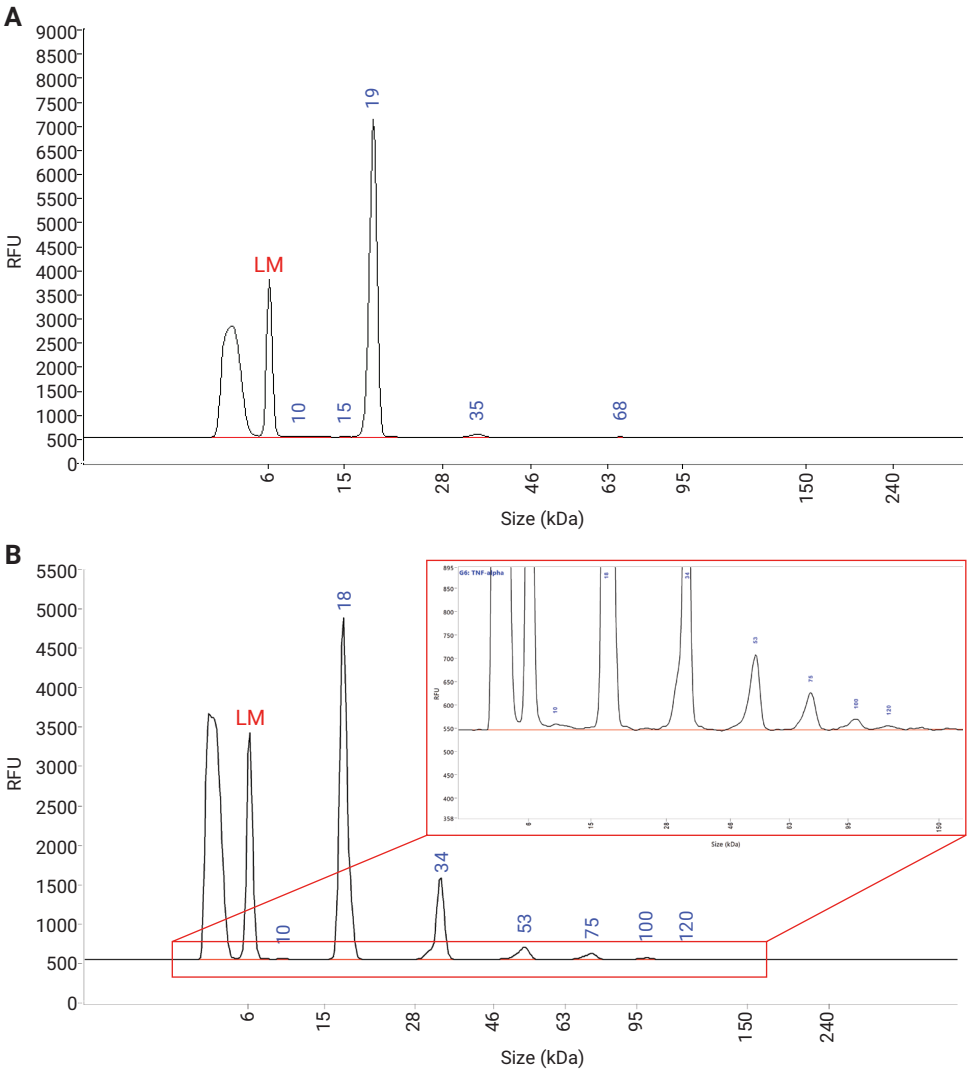

Figure 2. Analysis of the A) reduced and B) nonreduced forms of TNF-alpha on the Agilent ProteoAnalyzer system.

Table 2. Purity analysis of growth factors and cytokines assessed on the Agilent ProteoAnalyzer system.

Protein	Conditions		Average Purity (% total)	Precision (%CV)
IL-18	Reduced		98.1	0.31
	Nonreduced	Monomer	88.0	0.24
		Dimer	9.2	0.00
		Trimer	1.4	10.66
VEGF-165	Reduced		96.9	0.80
	Nonreduced	Monomer	95.9	0.48
		Dimer	2.2	16.39
		Trimer	0.7	7.87
TNF-alpha	Reduced		96.6	1.10
	Nonreduced	Monomer	75.3	0.73
		Dimer	17.4	1.20
		Trimer	3.8	3.98
		Tetramer	1.9	2.99
		Pentamer	0.8	21.65
IL-15	Reduced		86.8	1.13
	Nonreduced	Monomer	98.3	0.54
		Dimer	1.7	31.13

Conclusion

This application note highlights the sizing and purity analysis achieved by the ProteoAnalyzer system when analyzing a variety of cytokine and growth factor samples. Compared to traditional SDS-PAGE, the ProteoAnalyzer offers enhanced sensitivity, accurately detecting monomeric, dimeric, and trimeric forms of proteins, as well as small fragments that SDS-PAGE cannot visualize. This increased sensitivity is crucial for detailed protein analysis and helps to ensure reliable downstream results. The system offers highly accurate and reproducible sizing and precise purity measurements, underscoring its robustness and reliability. The ProteoAnalyzer system enhances the quality and reliability of protein analysis, offering detailed insights that are crucial for advancing research.

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www.agilent.com/genomics/proteoanalyzer

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