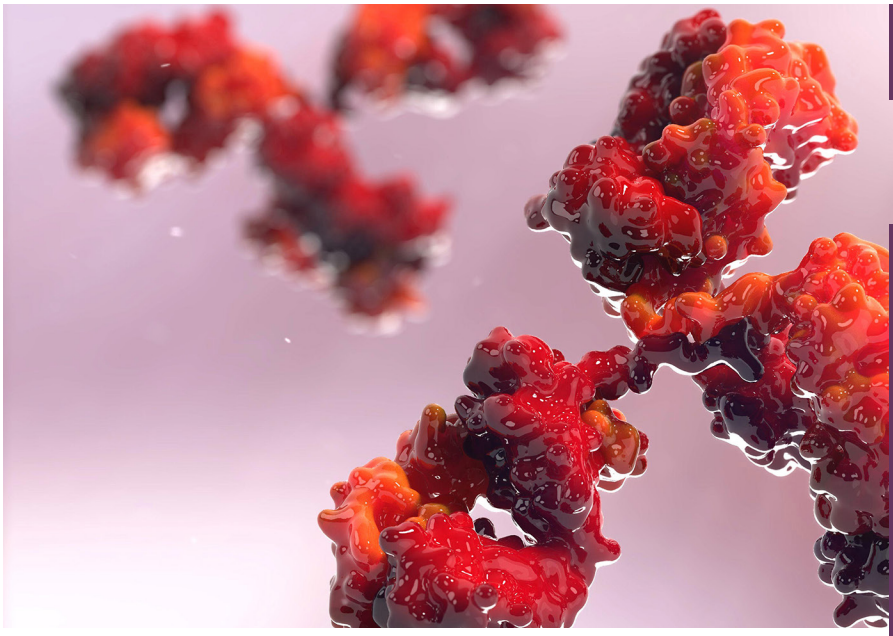

APPLICATION NOTE

Protein Clear™ HR Assay

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Independent Comparison of Protein Quality Control Analysis; an Evaluation of System Performance: LabChip® GXII Touch™ Protein Clear HR Assay v SCIEX® PA800 Plus System

Introduction

The LabChip® GXII Touch™ Protein Clear™ HR assay enables high-throughput microfluidic electrophoresis analysis of mAb samples. Inter/Intra-run reproducibility of mAbs in both non-reducing and reducing conditions, sizing, and stability indicating property are critical assessment criteria. Here we present data collected by an independent customer, in which mAbs were evaluated using both the Protein Clear™ HR assay and an orthogonal method, the SCIEX® PA800 Plus system.

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Assay Overview and Experimental Setup

The Protein Clear™ HR assay performance was compared to SCIEX® PA800 Plus system (also a capillary electrophoresis assay) using commercially available mAbs (BOC SCI, New York). mAbs were tested multiple times (N4) in the same run and also tested on the subsequent day using both assays to measure inter-run variability. Identical samples were tested with both assays.

Sample Preparation

Test IgGs included adalimumab (Ada) and infliximab (Inf), in both non-reducing (NR, iodoacetamide) and reducing (R, dithiothreitol) conditions. The adalimumab was also thermally stressed to induce a small amount of degradation. The adalimumab sample (0.5 mL aliquot, 10 mg/mL) was separated into 0.25 mL fractions. One fraction was stored at 4°C. The second fraction of adalimumab sample was heated at 55°C for 5 days in an Eppendorf® low bind tube for the stressed testing condition. Post-heating, the adalimumab was stored at 4°C until testing. The infliximab sample (0.25 mL aliquot, 10 mg/mL) was simply thawed and stored at 4°C until testing.

Assay Conditions

Protein Clear™ HR Assay Samples –10 µL of the stock IgG (10 mg/mL) was mixed into 90 µL of PBS to give 100 µL of 1 mg/mL IgG sample. The assay was run according to manufacturer's instructions. For this, 5 µL of IgG is mixed with 35 µL of either reducing (DTT) or non-reducing sample buffer and the mixture is heated at 70°C for 10 minutes. Following heating 70 µL of Millepore Sigma Milli-Q® water is added to the sample to give a 110 µL total. Approximately 30 µL of sample was used for each measurement.

SCIEX® PA800 Plus System Samples – 10 µL of 10 mg/mL stock IgG was used for each injection on the SCIEX® PA800 Plus system. Approximately 10 uL of IgG (10 mg/mL) was mixed with sample buffer (~90 µL) for each measurement.

IgG Measurements (Completed on subsequent days)

Assay	Test Sample	Stress Condition	Oxidation Status	Replicates
PA800	Adalimumab	Non-stressed	Non-reduced	4
PA800	Adalimumab	Non-stressed	Reduced (DTT)	4
PA800	Infliximab	Non-stressed	Non-reduced	4
PA800	Infliximab	Non-stressed	Reduced (DTT)	4
PA800	Adalimumab	Stressed	Non-reduced	4
PA800	Adalimumab	Stressed	Reduced (DTT)	4

Assay	Test Sample	Stress Condition	Oxidation Status	Replicates
Protein Clear™	Adalimumab	Non-stressed	Non-reduced	4
Protein Clear™	Adalimumab	Non-stressed	Reduced (DTT)	4
Protein Clear™	Infliximab	Non-stressed	Non-reduced	4
Protein Clear™	Infliximab	Non-stressed	Reduced (DTT)	4
Protein Clear™	Adalimumab	Stressed	Non-reduced	4
Protein Clear™	Adalimumab	Stressed	Reduced (DTT)	4

Results

Reproducibility

The intra- and inter-run precision for non-reduced and reduced samples were compared for each assay type. Each sample type was measured with 4 replicates on 2 days. The average standard deviation for the percent purity measurement during an experiment with (N4) for each sample type is shown in Figure 1. The Protein Clear™ HR assay showed variation that was comparable or lower than the SCIEX® PA800 Plus method. Each assay afforded a stable signal with good baseline for a given run. The inter-run precision (CV) are shown in Table 1. For each sample tested, the Protein Clear™ HR assay afforded a lower inter-run CV than observed for SCIEX® PA800 Plus system.

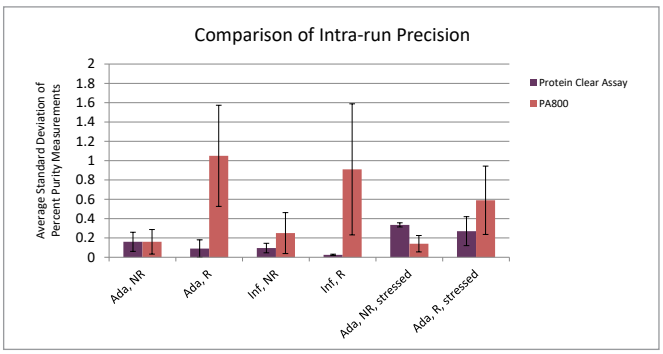

Figure 1. Comparison of the reproducibility of the Protein Clear™ HR assay and SCIEX® PA800 Plus system. The Protein Clear™ HR assay afforded standard deviation less than 0.5% reported on specifications across all samples tested.

Table 1. Inter-run precision (CV) for non-reduced and reduced IgG samples. The reduced adalimumab samples used manual integration to include some degradation peaks.

Sample Type	Protein Clear™ HR Assay	PA800 Plus
Adalimumab, NR	0.16	0.18
Adalimumab, R	0.27	1.52
Infliximab, NR	0.22	0.38
Infliximab, R	0.03	1.44
Adalimumab, NR, Stressed	0.34	0.97
Adalimumab, R, Stressed	0.16	2.74

Sizing

The protein sizing was also compared for each assay type, with results shown in Figure 2. Significant differences in sizing were made depending on the assay used. The non-reduced adalimumab and infliximab molecular weights are both ~144 kDa. The Protein Clear™ HR assay reported sizing that was in better concordance with the true size of the IgG molecule. The inter-run sizing CVs are shown in Table 2. The Protein Clear™ HR assay inter-run sizing CV was < 1%, whereas PA800 plus inter-run sizing CV could be significantly higher than the 1%. The electropherograms for non-reduced infliximab are shown in Figure 3. Markedly, the Protein Clear™ HR assay electropherogram is obtained in 65 seconds versus 40 minutes for SCIEX® PA800 Plus system. The Protein Clear™ HR assay shows aligned arrival time of symmetrical IgG sample peaks with high baseline quality.

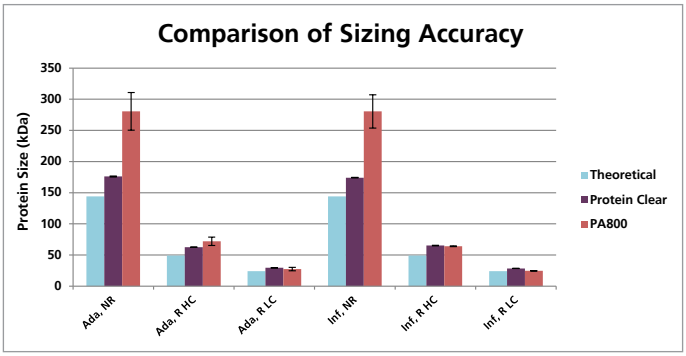

Figure 2. Comparison of sizing (kDa) output for Protein Clear™ HR assay and SCIEX® PA800 Plus system. The Protein Clear™ HR assay afforded greater accuracy for non-reduced mAb samples, and comparable accuracy for reduced mAb samples. The standard deviation of the sizing measurement was lower for Protein Clear™ HR assay than for SCIEX® PA800 Plus system.

Table 2. Sizing CV comparison for non-reduced and reduced IgG samples.

Sample Type	Protein Clear™ HR Assay	PA800 Plus
Adalimumab, NR	0.86	8.62
Adalimumab, R LC	0.71	26.39
Adalimumab, R HC	0.88	26.4
Infliximab, NR	0.77	7.48
Infliximab, R LC	0.85	3.73
Infliximab, R HC	0.78	2.66
Adalimumab, NR, Stressed	0.78	11.05
Adalimumab, R, Stressed LC	0.65	28.52
Adalimumab, R, Stressed HC	0.72	27.83

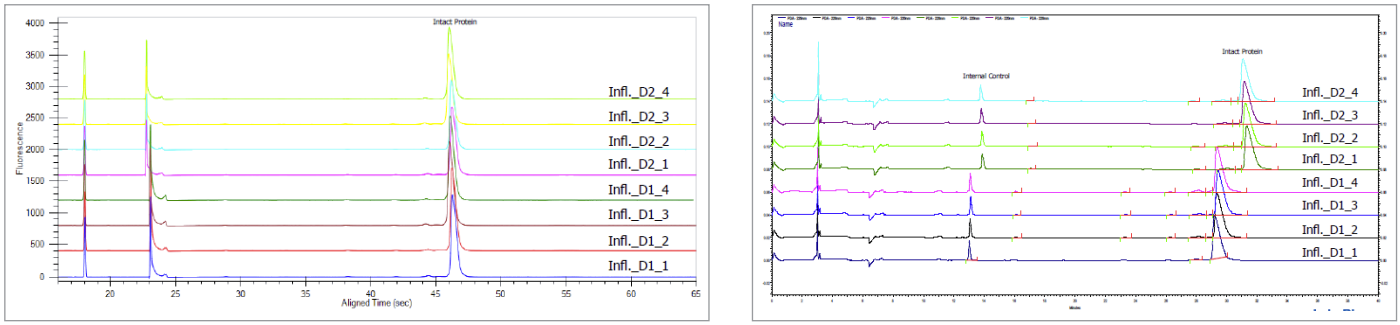

Figure 3. Comparison of electropherograms on non-reduced Infliximab for each assay. **Left:** The Protein Clear™ HR assay results showed improved peak symmetry, aligned arrival time, and good baseline quality. **Right:** Electropherogram results for the same non-reduced infliximab samples obtained using the SCIEX® PA800 Plus system.

Conclusion

In this technical note, we present data that the PerkinElmer® Protein Clear™ HR assay affords percent purity measurements at a precision level that is comparable to SCIEX® PA800 Plus system. In addition, the Protein Clear™ HR assay was significantly better at measuring protein size and was also able to detect mAb purity using the assay ladder. The high reproducibility of this assay advances microfluidic capillary electrophoresis technology to out-perform in rigorous quality control groups.

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