

**LabChip® GXII Touch™ Protein Characterization System****Authors**

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## ProteinEXact™ Assay

### Introduction

The LabChip® GXII Touch™ protein characterization system performs microfluidic capillary electrophoresis ( $\mu$ CE-SDS) with throughput flexibility for protein analysis. The ProteinEXact™ assay was designed to improve sizing range for protein analysis and improve the concentration CV measurement to ~10% CV. Assay capabilities for the ProteinEXact™ assay include: 1) protein sizing ladder ranging from 6.5 - 250 kDa that is separated on the PerkinElmer high resolution chip and 2) ProteinEXact™ IntelliChip™ assay optimization technology for precision enhancement. The software interface allows the user to enter a lot specific concentration of the ladder. Using this concentration standard, the ProteinEXact™ IntelliChip™ assay optimization technology optimizes the laser strength (mW) to report the ladder concentration with the highest concordance to the theoretical value.

## Experimental Design

A panel of proteins ranging from low molecular weight (Aprotinin, 6.5 kDa) to highest molecular weight (EP, 250 kDa) was prepared at 1000, 500, 100, and 10 ng/μL. Infliximab (non-reducing condition and reducing condition) was used as a representative monoclonal antibody. All proteins were prepared in phosphate buffered saline solution prior to assay analysis.

The ProteinEXact™ assay was run according to the manufacturer's instructions. Samples denatured under non-reducing conditions were mixed at the following ratios: 5 μL of sample was mixed with 35 μL of non-reducing sample buffer that was prepared by combining 700 μL protein sample buffer with 24.3 μL of iodoacetamide (250 mM in water). Samples denatured under reducing conditions were mixed at the following ratio: 5 μL of sample was mixed with 35 μL of reducing buffer prepared by combining 700 μL protein sample buffer with 24.3 μL of DTT (1M in water). The samples were heated at 70°C for 10 minutes followed by addition of 70 μL of Millipore® Milli-Q® water. Each sample was prepared on 9 separate plates. The plates were measured on three LabChip® GXII Touch™ protein characterization systems by four different users. Each sample type was sipped four times (N4) during a run, with inter-run sizing and concentration measurements representing 54 sips total for each protein.

## Results and Discussion

### ProteinEXact™ Ladder

An electropherogram of the ProteinEXact™ ladder is shown in Figure 1 which illustrates the expansive range for concentration and sizing detection.

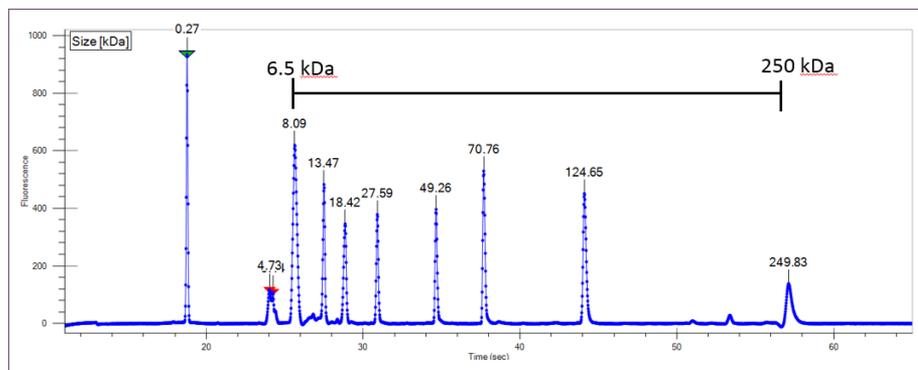


Figure 1. ProteinEXact™ assay ladder with peaks ranging from 6.5 kDa to 250 kDa peaks.

### Sizing CV

The sizing CV for the protein samples is shown in Figure 2. The ProteinEXact™ assay has a specification of 2% for sizing CV. All samples passed this criterion, and 19/21 samples (90%) showed a CV of less than 1%. The ProteinEXact™ assay uses constant current for the electrophoresis. Alignment to the lower marker allows for increased assay precision for sizing.

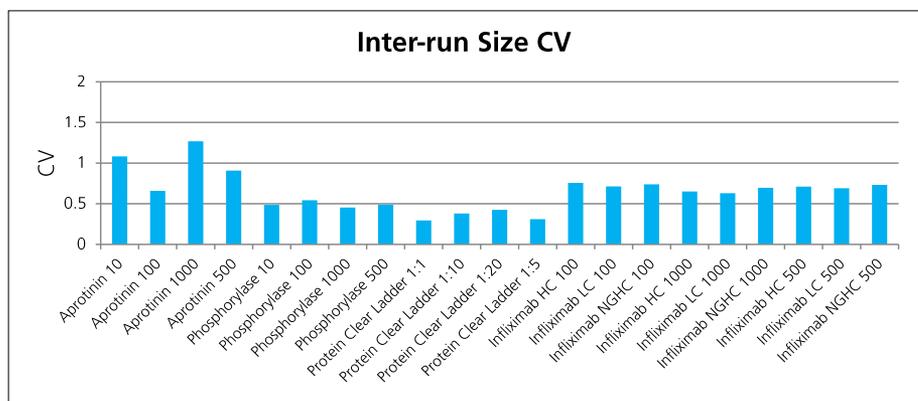


Figure 2. Sizing CV for ProteinEXact™ Assay

### Concentration CV

The concentration CV for the protein samples is shown in Figure 3. The ProteinEXact™ assay introduces a laser powered optimization to minimize concentration CV. Robustness of the assay for determination of concentration is further demonstrated with the reproducibility in high and low concentration sample results in Figure 4.

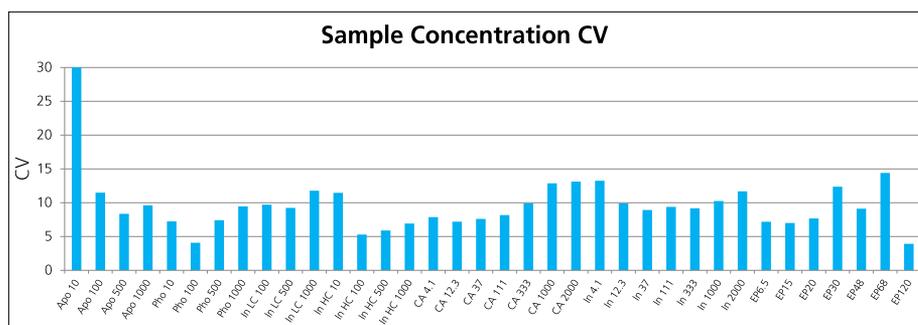


Figure 3. Concentration CV for the ProteinEXact™ assay

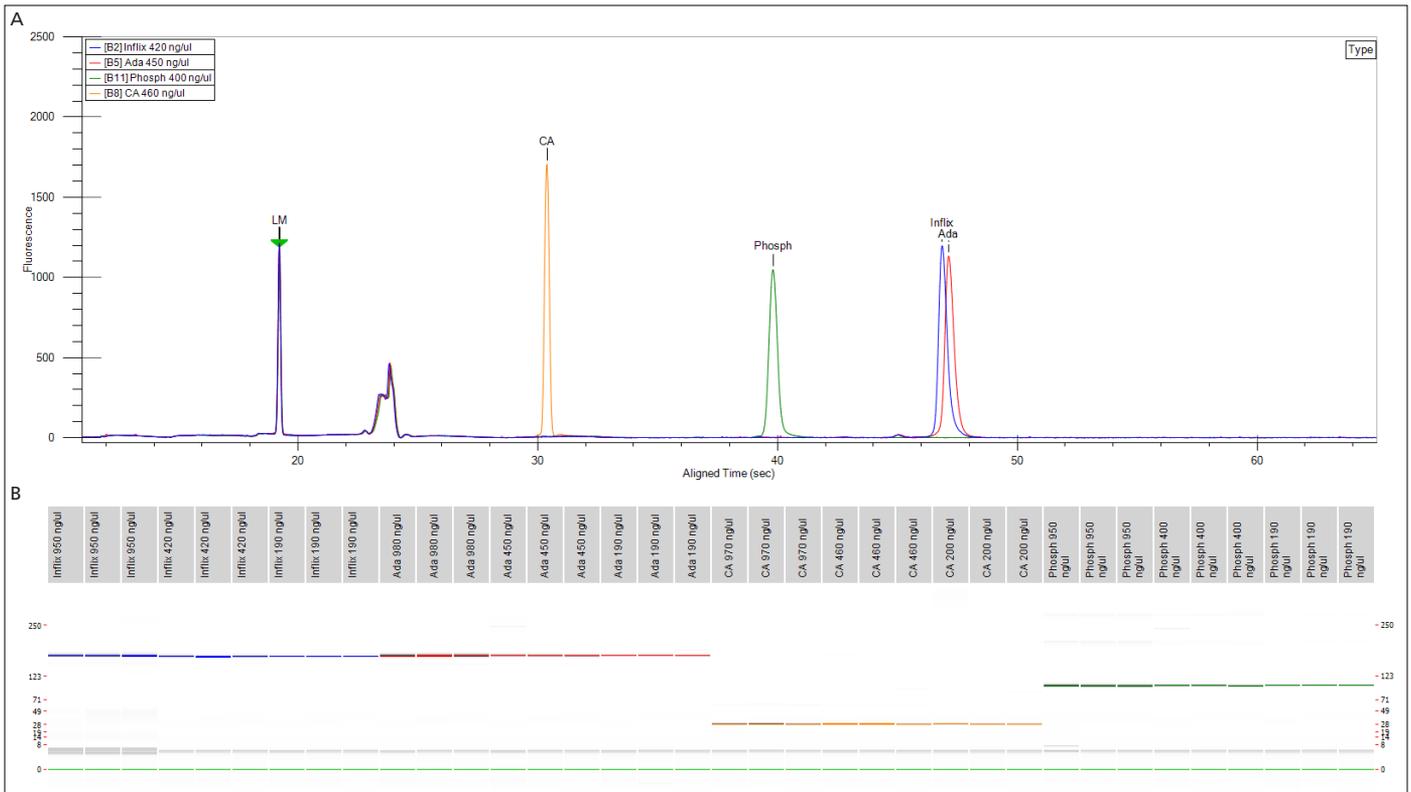


Figure 4. Electropherograms show distinctly resolved proteins (a) and the associated virtual gels (b) demonstrate reproducibility at both high and low concentrations.

### Concordance to UV Measurements

The concordance was also analyzed by comparing concentration measurements made using the ProteinEXact™ assay with those measured using UV spectrophotometry using the same samples (Figure 5). A new sample plate containing carbonic anhydrase (CA), phosphorylase (Phos), Adalimumab (Ada), and Infliximab (Infl) at concentration 200, 500, and 1000 ng/μL. For each protein, the sample was measured in duplicate using the DropletQuant® spectrophotometer and the ProteinEXact™ assay. To increase concentration accuracy, an optimization of the protein sizing correction factor was applied according to our Technical Note.<sup>1</sup> The difference from the theoretical UV absorption measurement ranged from 0.003 - 0.19 ng/mL\*, with average difference 0.14 mg/mL.

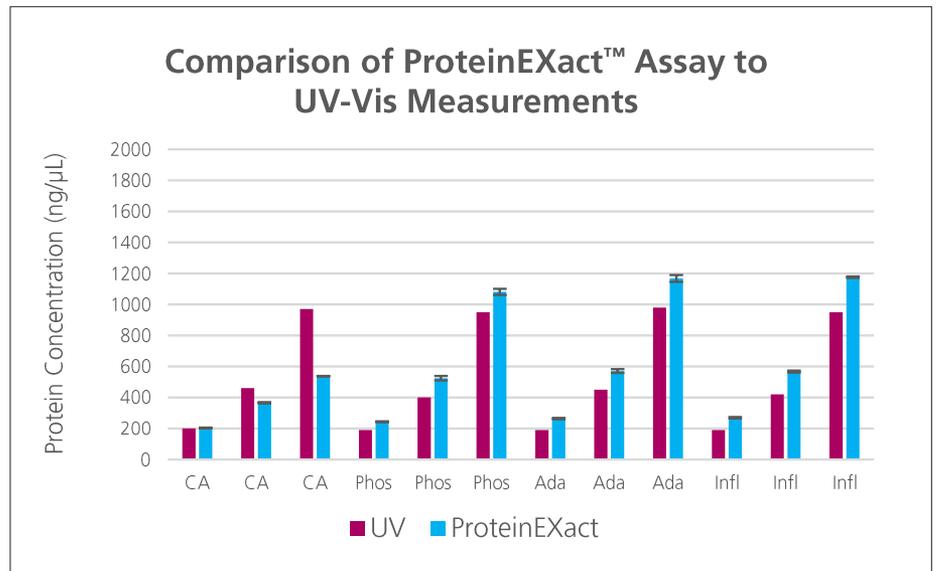


Figure 5. Comparison of ProteinEXact™ assay to UV-Vis measurements. \*Carbonic anhydrase at 950 ng/μL appears to be an outlier

## Conclusion

Here we demonstrate expected performance of the ProteinEXact™ assay. The expansive sizing range allows for detection of both small molecular weight products and degradation products. Meanwhile, a 250 kDa ladder peak is included for bracketing monoclonal antibody samples (MW ~ 150 kDa). The ProteinEXact™ IntelliChip™ assay optimization technology enables higher precision measurements of protein concentration. The ProteinEXact™ assay used with microfluidic capillary electrophoresis is an optimized technique for rapidly assessing sizing and concentration of protein samples. For industrial protein scientists involved in mAb production and protein purification science the assay also simplifies percent yield (concentration). In addition, the ProteinEXact™ assay serves as a new tool for measuring the controlled release (concentration) of biotherapeutics from biomaterials and would eliminate time consuming ELISA assays for this line of research and development.

## Reference

1. Protein Quantitation Methods using LabChip GX Touch Platform, Technical Note, James White, Zhiyong Peng, Natalia Rodionova, Rachel Gelineau, Erik Miller, 2018

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