

New Series II Protein 230 assay for the Agilent 2100 bioanalyzer

Technical Overview

Introduction

This Technical Note compares the new Protein 230 assay with its predecessor, the Protein 200 Plus assay. The superior Protein 230 kit replaces the Protein 200 Plus kit, which will be obsolete by the end of June 2006.

The main improvements of the Protein 230 assay are:

- Extended size range
- Improved specifications on sizing accuracy, sizing reproducibility and quantitation reproducibility
- Exceptional purity and stability of ladder and sample buffer
- Higher sensitivity
- Stable and reproducible baseline

Sizing

In comparison to the previous Protein 200 Plus kit, the series II Protein 230 kit offers an extended sizing range. It is now possible to confidently analyze proteins ranging from 14 to 230 kDa. To achieve this a new ladder consisting of six recombinant proteins, as well as a new lower (4.5 kDa) and upper marker with a calculated electrophoretic mobility of 240 kDa, were developed (figure 1).



The Protein 230 ladder peaks are more evenly spread throughout the size range and now include a protein at 150 kDa providing higher confidence in sizing. In addition, the new ladder and the upper marker have a significantly higher purity (>98%) improving the accuracy and robustness of the automated sizing (figure 2). For samples containing bovine serum albumin (BSA) or carbonic anhydrase II (CAII) in phosphate buffered saline (PBS) a sizing accuracy below 10 % and a reproducibility under 3 % CV was achieved.

Lower and upper marker

The new upper marker, a recombinant protein, replaces the less stable myosin (calculated electrophoretic mobility of 210 kDa) and allows extending the size range beyond the previous 200 kDa to 230 kDa. There is no detectable impurity in the electropherogram (S/N>3), when analyzing a blank sample (figure 3), making this assay especially useful for purity determination of column fractions and other protein samples. The purity of the previous Protein 200 Plus sample buffer was only 95 % with two visible impurities of 18 and 25 kDa.



Figure 1

Protein 230 ladder. The ladder consisting of six recombinant proteins and an upper and lower marker is used for the sizing of unknown protein samples. The gel-like image and the electro-pherogram are shown.





Samples containing various concentrations of BSA or CAII were analyzed using the Series II Protein 230 assay. The peak height is plotted against the peak size to determine sizing accuracy and reproducibility, independently of the protein concentration.



Figure 3

Purity of the Protein 230 upper marker. The analysis of a blank sample (PBS) demonstrates the purity of the sample buffer. There are no detectable impurities in the electropherogram of the Protein 230 sample buffer. The complete electropherogram is shown in A and a baseline zoom in B.

The new lower marker is a fluorescent dye which migrates at 4.5 kDa. This ensures a greater distance between system peak and lower marker as compared to the previous lower marker, which migrated at 6 kDa. Therefore, a safe automatic identification by the Agilent 2100 expert software is guaranteed. In addition, the lower marker is more stable in the presence of detergents and high salt concentrations in the sample matrix.

Both the Protein 230 ladder and the markers are also used for the Protein 200 HT-2 assay on the Automated Lab-on-a-Chip Platform (ALP). This allows for a more efficient comparison of results and application transfer between the two lab-on-a-chip platforms, the Agilent 2100 bioanalyzer and the 5100 ALP.

Sensitivity

Further optimizations of the sample buffer, ladder, and dye-concentrate lead to a very stable baseline, allowing detection of as little as 15 ng/µL BSA and 6 ng/µL CAII in PBS with a S/N (signal to noise) level above 3 (figure 4). These modifications provide a very reproducible protein quantitation in buffers with low ionic strength or even in water.

Quantitation

The new Protein 230 kit not only includes changes in the reagent composition, as described above, but also adaptations in the assay script. These modifications result in a higher reproducibility of the injection of the upper marker, which directly translates into a much more reliable quantitation. The automated quantitation is based on the upper marker area relative to the sample. Moreover, it is possible to use ex-ternal standards to achieve an absolute quantitation. Additional modifications in the script, combined with the buffer adjustment in the reagent kit, further improve the baseline stability and reproducibility.



Figure 4

Sensitivity of the Protein 230 assay. Samples containing 6 ng/µL CAII in PBS were analyzed using the Series II Protein 230 assay. The full electropherogram is shown in A, and the enlargement of the CA II peak in B (S/N ~ 6).

Kit components and ordering

All Protein 230 kit components are orderable under one part number and are now conveniently delivered in separate boxes according to their storage conditions (table 1). As previously, the reagents can also be ordered separately. The lifetime of the Protein 230 reagents was extended to a minimum of four months if stored appropriately. Additional tests are being performed to further extend lifetime.

With the above outlined modifications the Protein 230 assay provides a significant improvement in performance and specifications over the Protein 200 Plus assay (table 2).

Complete kit (5067-1517)			
Protein Chips (storage at room temp.)	Reagents (5067-1518)		
	Protein 230 Reagents Part I (storage at 4 °C)	Protein 230 Reagents Part II (storage at -20 °C)	
Protein chips Electrode cleaner	Gel matrix Dye concentrate Spin filters	Ladder Sample buffer	

Table 1

Protein 230 kit components.

	Protein 200 Plus	Protein 230
Sizing range	14-200 kDa	14-230 kDa
Sizing resolution	\pm 10% across sizing range	No change
Typical sizing accuracy		<i>10 % *</i>
Sizing reproducibility		3 % CV *
Sensitivity (S/N>3)	20 ng/uL BSA	6 ng/µL CAII in PBS
		15 ng/µL BSA in PBS
	40 ng/µL BSA in 0.5 M NaCl	30 ng/µL BSA in 0.5 M NaCl
Quantitative range	20-2000 ng/µL	6-2000 ng/µL CAII in PBS
		15-2000 ng/µL BSA in PBS
Qualitative range	20-4000 ng/µL	6-5000 ng/µL CAII in PBS
		15-5000 ng/µL BSA in PBS
Quantitation reproducibility		20 % CV *
Compatible buffers		See "List of Compatible
		Buffers and Buffer Compounds"
		located in the kit guide.

Table 2

* for typical proteins like BSA and CAII

Protein 230 Specifications. Improvements in specifications as compared to the previous Protein 200 Plus assay are highlighted.

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