

# **High Sensitivity SDS-protein Separations by Capillary Electrophoresis**

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### Abstract

Conventional electrophoresis (CE) of SDS-protein complexes is performed on a 2-dimensional slab, gel manufactured from polyacrylamide. This allows the on-gel staining of the separated protein complexes using such techniques as silver staining which although offering the highest sensitivity is, however, not quantitative. At best this technique is semi-quantitative due to the staining and detection (densitometry) techniques used to quantitate the separated bands. Capillary electrophoresis is inherently a quantitative instrumental method of analysis. CE may be used in conjunction with replaceable gel matrices in order to separate SDS-protein complexes with high resolution.

### **Experimental**

All experiments were performed using the Agilent Capillary Electrophorsis system equipped with diode array detection and computer controlled via Agilent ChemStation software. The instrument was fitted with the Agilent CE high sensitivity detection cell offering an increased pathlength of 1.2 mm and increased linear range to approximately 1300 mAU with less than 1 % deviation from linearity.

The capillary was filled with the sieving gel using the internal 1 bar pressure of the Agilent CE system. The time taken to fill and replace the capillary contents was approximately 10 minutes.





Figure 1 illustrates the increases in sensitivity obtained over the supplied BioRad SDS-protein kit

# **Conditions**

**Capillary** 400 mm (485) × 75 µm i.d. Buffer BioRad SDS-protein sieving buffer Injection 5 s at -10 kV **Run** -15 kV **Temperature** 40 °C **Detection** 220 nm/30 nm high sensitivity detection cell



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using the Agilent CE high sensitivity detection cell. In comparison to the results obtained from the 50  $\mu$ m i.d. capillary used in the BioRad kit the signal to noise was increased by a factor of approximately 8.5 for each peak. A graph of log MW versus migration time relative to that of the reference peak (benzoic acid) provides a linear relationship with  $r^2 = 0.9988$  (figure 2).

#### Conclusions

The Agilent CE high sensitivity cell can be used in for capillary gel electrophoresis experiments. In conjunction with bare fused silica capillaries, increases in sensitivity of over 8-fold can be achieved for SDS-protein determinations.



### Figure 2

SDS-protein capillary gel electrophoresis with high sensitivity cell

## Equipment

- Agilent Capillary Electrophoresis System
- Agilent CE high-sensitivity detection cell
- Agilent ChemStation + software



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