COMPARISON OF SDS-PAGE TO AUTOMATED PARALLEL CAPILLARY ELECTROPHORESIS FOR ENZYME SIZE AND PURITY ASSESSMENTS

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Traditionally, purified enzymes have been analyzed using SDS-PAGE to confirm the presence of the correct manufactured products and identify any impurities present in the sample. While this technique is widely used it is also manual, time consuming, and requires conversion into a digital format for accurate and thorough analysis. Staining procedures for traditional SDS-PAGE, such as Coomassie blue, further hamper purity assessments due to their limited quantitative linear dynamic range. With the digitization of laboratory records becoming more common and increasingly stringent quality standards, alternatives to SDS-PAGE are needed. To address these problems with SDS-PAGE, Agilent has developed a 12-channel, parallel Capillary Electrophoresis-SDS (CE-SDS) instrument and reagents. Samples are prepared directly in a 96-well plate and covalently labeled with a fluorescent dye allowing for a 3-log dynamic range. The system automatically cleans and conditions the capillary environment prior to filling with fresh sieving gel to prevent protein carryover and buildup. Internal standards allow precise sizing through 240 kDa and quantification/purity results for a variety of reduced and non-reduced proteins. This automatic cleaning procedure allows for the analysis of samples ranging from crude cell lysates to purified enzymes. The data can be individually analyzed as a high-resolution electropherogram and as a digital gel image for quick comparisons of multiple samples. This poster provides sizing and purity comparisons between the new Agilent ProteoAnalyzer system and SDS-PAGE using commercially available DNA Polymerases, Restriction Enzymes, and other purified proteins. The system demonstrates equivalent sizing to traditional SDS-PAGE while also providing impurity detection down to 0.1% due to the increased quantitative dynamic range.