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A comparative study of commercially synthesize immobilized pH gradient (IPG) strips for two-dimensional electrophoresis Lindsay Freeberg and Jay Thelen

Two-dimensional electrophoresis (2-DE) is a widely used technique for resolving complex protein mixtures. The resurgence in this technique can be attributed to the contemporary approach for isoelectric focusing, which relies on an immobilized pH gradient of ampholines embedded within an acrylamide matrix. Furthermore, the commercial production of these Immobilized pH Gradient (IPG) strips has further simplified this technique to improve consistency of 2-DE. Since IPG strips are primarily purchased from vendors, rather than synthesized by the investigator, we wanted to compare the efficacy of strips from the principal manufacturers. In this study IPG strips selected from three major life science companies, Amersham, Bio-Rad, and Sigma (produced by Proteome Systems) were quantitatively compared for linearity of signal (spot volume) and qualitatively for parameters such as spot shape and same-sample reproducibility. To control for sample variation a *Brassica napus* protein leaf extract, prepared using an established protocol, was used as the test sample for all experiments. To compare the protein loading capacity and linear quantitative range, IPG strips were loaded with four different concentrations of protein: 50g, 100g, 200g, and 300g using the isoelectric focusing protocol recommended for Amersham strips. However, since the focusing protocol recommended for the Sigma IPG strips was approximately 8,000 Volt hours longer we also tested the strips at a longer focusing protocol. Spot volume was quantified using Phoretix 2D Advanced software. The spot volume was then plotted on graphs to determine the linear quantitative range. The graphs show Sigma to have the best quantitative range for each spot. However, Amersham IPG strips yielded more circular spot shapes and were more reproducible on a lot number and day-to-day basis.