Large-Gel 2-D Electrophoresis (carrier ampholyte technique)

Dijana Sagi
Institut für Humangenetik, Charité - Universitätsmedizin Berlin

Two-dimensional gel electrophoresis is the most widely used protein separation technique in proteomics, due to its good resolving power and ability to separate protein isoforms. There are two different 2-D electrophoresis (2-DE) techniques: immobilized pH gradient-based and carrier ampholyte-based 2-DE. The latter will be presented here. The main feature of the presented 2-DE technique is the use of large gels with a resolving power of more than 8000 protein spots. The technique includes the preparation of 40-cm IEF gels in capillary tubes, cutting the long gels into two halves after the IEF run, transfer of the two half-gels onto two normal-sized SDS gels with a running distance of 30 cm, staining (silver) of the separated spots and drying the 2-D gels. Among the technical details and special equipment which constitute this 2-DE method, a few features are of particular significance: (1) sample loading onto the acidic side of the IEF gel with the result that both acidic and basic proteins are well resolved in the same gel; (2) use of large (and thin) gels to achieve high resolution and (3) preparation of ready made gel solutions which can be stored frozen, a prerequisite, among others, for high reproducibility. Due to this features we use large-gel 2-DE with carrier ampholytes for the separation of protein samples of high complexity, e.g. whole brain or liver tissue.