

2-Dimensional gel electrophoresis (2DE) is a low throughput but quantitative method for resolving protein mixtures on the basis of isoelectric point in the first dimension and molecular weight in the second. This method is useful for identifying and quantifying subsets of proteins differing between complex biological samples. For example, protein kinases are known to control cell division and are involved in the unregulated cell growth of cancer. The subset of proteins phosphorylated by tyrosine kinases in tumors are of great interest. Although 2D gel staining methods are relatively insensitive (ng- μ g level), immunostaining by Western blotting with antibodies against specific proteins increases sensitivity 10-100 fold. Results of 2D gel quantification with Progenesis Discovery software and Coomassie blue staining will be presented as evidence of method reliability for the carrier ampholine method of IEF. Results of phosphotyrosine 2D Western blotting will be shown along with advances in the more difficult area of phosphoserine and phosphothreonine immunostaining. The new and highly sensitive ECL Advance reagent from GE Healthcare has facilitated Western blotting for the latter.