

A COMPARISON OF SILVER, SYPRO RUBY AND RUTHENIUM II TRIS STAINS FOR 2D-GEL ELECTROPHORESIS WITH RESPECT TO IMAGE ANALYSIS AND PROTEIN IDENTIFICATION

Marisol Fernández, Sergio Ciordia, Silvia Juárez and Juan Pablo Albar

Proteomics Facility, Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Proteomic projects are often focused on the discovery of differentially expressed proteins between control and experimental samples. One approach is running two-dimensional gels, analyzing them and identifying the differentially expressed proteins by tryptic in-gel digestion and mass spectrometry. Two restrictive factors for this type of proteomic mapping are the sensitivity of the staining technology and the recovery of tryptic peptides for mass spectrometry identification. In this report, we compare two fluorescent dyes: SYPRO-R and RuBP versus silver staining procedures with respect to several parameters: sensitivity, amount of proteins, number of spots detected by image analysis and peptide recovery after in-gel protein digestion for matrix-assisted laser desorption/ionization mass spectrometry. 100 µg of total protein extracts obtained from 293T cells were loaded and fractionated in 2D-PAGE gels and the scanned images analyzed with the Image Master 2D Platinum software. Proteins were excised, automatically digested under the same conditions with trypsin and the resulting peptides were extracted and analyzed by MALDI-TOF/TOF mass spectrometry. All three staining procedures showed advantages and disadvantages. Silver staining is more sensitive on the number of spot detected and allows a greater peptide recovery and therefore identification of lower abundance proteins but SYPRO-R reports higher protein scores when MS/MS analyses are performed. The RuBP procedures give similar contrast and detection than SYPRO-R but it is a very large method.