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DEVOLVER AO BALCÃO DE EMPRÉSTIMO

QUANTITATIVE PROTEIN ELUTION FROM UNSTAINED POLYACRYLAMIDE GELS

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Many works have described the extraction of proteins from gels after running Polyacrylamide gel electrophoresis (PAGE). Only some of them used unstained gels, exploring direct UV densitometry which does not alter the biological properties of the product. Very few of these, though, describe quantitative recoveries, being often satisfied with 70-80% yields and most of the times using very costly and complicated machineries or time consuming multiple-step techniques.

In previous work we set up a simple (single technique) and rapid (~6 h.) preparative technique via reversed electrophoresis elution and we demonstrated that the whole process does not alter the somatotrophic activity of hGH (Arq. Biol. Tecnol. 1985: 28: 168-0 16). In the present work we carried out an extensive study in order to find out the causes of protein losses, trying then to modify the technique so to obtain quantitative recoveries (90-100%). For this purpose monomeric bovine serum albumin (PSA) was run and eluted together with ~50 nCi of ¹²⁵I-BSA tracer, prepared in our laboratory. Radioactivity recoveries were checked after each operation in all materials and reagents used in the following three main steps: a) Resolving large-gel electrophoresis; b) Reversed electrophoresis; c) Analytical small gel electrophoresis (including dialysis).

Three experiments, carried out with 2 mg PSA + tracer, presented these yields: 97.3±1.8 % after step a; 71.3±4.9 % after step b; 49.8±4.0 % after step c. The major sources of protein losses were: reversed electrophoresis (26%), dialysis (7%) and final analytical step (14%). We decided therefore to increase the volume of elution buffer introduced into the dialysis sac (step b), to substitute the dialysis between step b and c with a simple sample dilution and to improve the analytical step (c) by the use of longer gels (11 cm) and of a higher amplification in the densitometric reading. PSA recoveries went from 69.4±11.1 % to 96.5±8.2 %. In the modified technique, instead of the usual 2 mg protein load, also 1.0 and 0.5 mg protein were applied to the large preparative gel, still with more than 90% recoveries.