THE COMPLETE SET OF TOOLS FOR PROTEIN TWO DIMENSIONAL ELECTROPHORESIS IN EIGHT SLABS OF POLYACRYLAMIDE GEL

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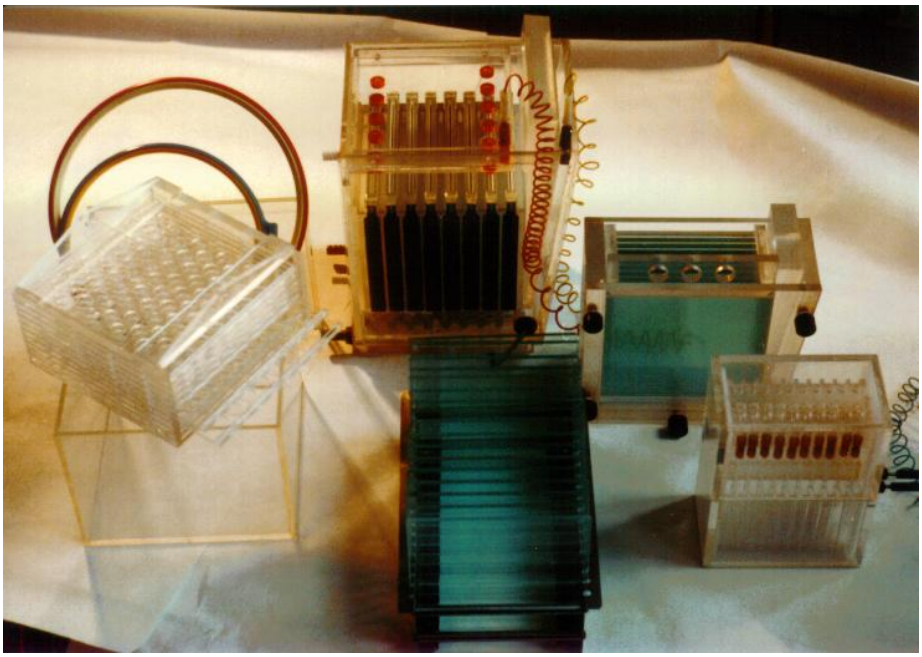


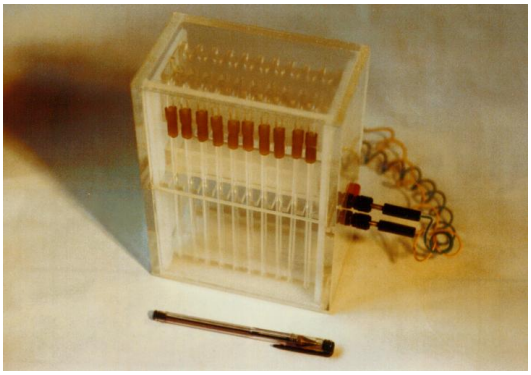
The set of tools was specially developed for proteomic researchers. It is intended for comparative analysis of complicated protein mixtures using synchronous two-dimensional gel electrophoresis in eight slabs of polyacrylamide gel. The design provides excellent reproducible separation of proteins. It greatly improves throughput and is easy in operation.

Existing and available two-dimensional gel electrophoresis systems provide synchronous analysis only of one or two samples. Low throughput, considerable labor and high cost are usual shortcomings. Besides, these systems lack special devices for gel washing, protein development and fixation, for gel drying. The suggested set gets over all these difficulties.

The investigation of silver staining mechanism opens the perspective of sensitive and reproducible quantitative analysis of the thousands of protein fractions from natural samples.

BRIEF DESCRIPTION OF ELECTROPHORETIC TOOL SET

1. Device for protein isofocusing in tubes (the first dimension)

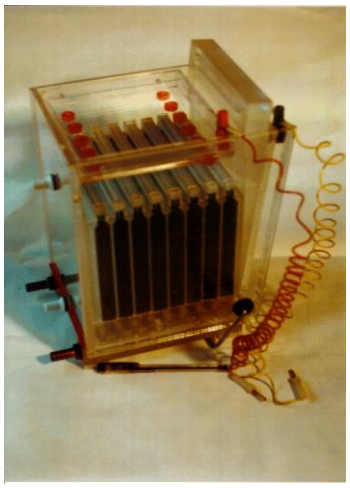


The system was elaborated to change glass tubes with gel on stream. It was achieved by using salt bridges between tube upper ends and upper reservoir. Salt bridge is a glass U-tube filled with upper electrolyte and fixed at tube upper end by means of rubber muff. The lower tube ends are sunken in lower electrolyte reservoir. Platinum electrodes are placed into the electrolytes. They are under the direct voltage. The system is placed into plexiglass box with removable cover for protection from CO_2 absorption. Besides direct and inverse isofocusing the device could be used for other gel electrophoresis kinds. The device simplifies the isofocusing procedure and guarantees reproducibility of results.

2. Device for synchronous preparation of eight gel slabs

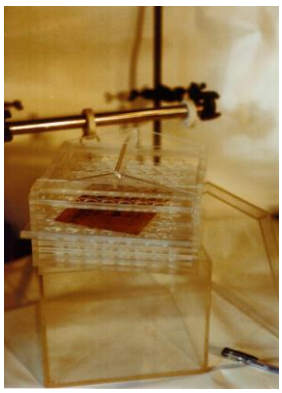
The device is a plexiglass cell with screw sidewall. Eight cassettes are placed into this cell. Each cassette is compiled from two glass slabs divided by two spacers (one on each side). At the cell bottom there is a plate with dichotomous branching grooves. Gel forming solution enters from the external connecting pipe through these grooves and is directed to a hole crossing cassettes in the middle of the cell bottom. From there it is divided among eight cassettes. Dichotomous groove branching provides fluid flow symmetry division. As a result high degree similarity of gel density gradient is in gel slabs. A special wedge is located inside the cell. After its pulling a cave appears. It releases the cassette edges and as a result makes easy the cassette extraction after gel polymerization. The device saves time and labor at slab preparation.

3. Device for protein electrophoresis in eight gel slabs (the second dimension)



The device is a cell divided by 8 flat heat exchangers. They are flexibly fastened at the cell bottom. Thermostating fluid circulates through these exchangers. These heat exchangers form 8 parallel wells. Eight cassettes with gel slabs are inserted upright into these wells. Heat exchangers and cassettes are pressed with the help of wedge to a close package. As a result two reservoirs are formed: one for upper and another for low electrode buffers. In case of two-dimensional electrophoresis gel columns after isofocusing are fixed on upper slab edges. In case of one-dimensional electrophoresis liquid samples are located into special holes on upper slab edges. Platinum electrodes placed into upper and lower reservoirs provide direct current passage. It results in protein separation. The device saves labor and guarantees similarity of protein fractionation.

4. Device for washing and development of eight gel slabs



The device is a plexiglass cell with a cover. Rectangular basket carrying 8 removable perforated shelves is put inside the cell. Extracted from cassettes after electrophoresis gel slabs are placed on the basket shelves. Then the basket is put into the cell containing washing or developing solutions. The shaking of solution is carried out by slow basket swing due to its connection with rotating eccentric shaft outside. The device saves time and guarantees stability of the results.

5. Gel dryer

Two hoops fasten two cellophane sheets around the perimeter with gel slab inside. This device provides gel drying during 4 –5 hours in hanging state at room temperature. This procedure is very simple and cost saving.

References

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