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- SPECIAL OFFER -

"TRIAL KITS" for Disc Electrophoresis are now being made up, so that you can try this remarkable new technique, at minimum cost, on your own samples. KIT consists of a complete set of chemicals for 100 experiments, plus all necessary equipment, accessories, and instructions. KIT can be used with any DC power supply which provides 20 ma at 150 V. Delivery to be made about July-August.

Price : TRIAL KIT, without power supply ... .. £ 17  
POWER SUPPLY MODEL 150 for  
Trial Kit ... .. £ 18

The above prices are net delivered U.K. destination. No allowance has been made for import duty since we are reasonably confident that duty free import can be arranged provided that a research basis exists with you and you are a non-profit making organisation.

The TRIAL KIT is a single-column instrument designed for easy prove-out of the system. It's resolution capability is slightly less than the Regular Unit, and it is not useful for handling any large volume of experiments, as each sample occupies the instrument for a total of about  $1\frac{1}{2}$  hours. By dovetailing operations, one could handle an absolute maximum of only 5 samples in an 8 hour day.

The Regular Unit is a twelve-column instrument offering the maximum resolution of the system, capable of handling up to 60 samples per day in the Single-Bath version, or 120 samples per day in the Double-Bath version.

The Regular Unit has been redesigned, and is being re-costed. Details will be forthcoming from the U.S.A. in a short while, but a large price reduction can be expected and we have received tentative details of a figure under \$400 without power supply (compared to the original \$960).

The TRIAL KIT will be advertised in a JUNE and a JULY issue of American SCIENCE, plus the JUNE "MEDICAL ELECTRONICS NEWS", and "JOURNAL OF CHEMICAL EDUCATION".

CANAL Disc Electrophoresis

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(6) How can separated materials be removed from the gel column?

- Fixed and stained proteins cannot be removed. Unfixed proteins can be removed in several ways:

- a. By slicing the gel column (preferably after freezing), pulverising the gel, and placing the pulverised gel in a suitable solution, (saline, distilled water, etc.) approximately of 4-5 times the gel volume. Protein will diffuse out in about three hours. Approximately 80% of the protein content can be captured in this way.
- b. By eluting out the bottom of the glass tube into a small dialysis bag. Fast fractions take about 1½ hours to reach the bottom.
- c. By electrophoretically eluting out the bottom of the glass tube into a short test tube submerged in the lower electrolyte. (Fraction collector).
- d. By electrophoresing pulverised gel sections into dialysis bags.

Comment: because unfixed proteins are also unstained, a "tracking dye" is provided which clearly shows the moving albumen and the salt "front" in serum runs. When eluting unfixed proteins, it is helpful to make a fixed-and-stained run first to use as a "road map". Unfixed fractions can be observed faintly at an angle against a light, because of their differences in refractive index from that of the gel. A simple Schlieren optical system is now under development to permit their easy recognition for control of elution and slicing techniques.

(7) Won't these unfixed proteins diffuse in the gel?

- Yes, that is why you should slice the section immediately after electrophoresis, or freeze it promptly in liquid nitrogen or dry ice.
- The rate of diffusion in the gel of unfixed components varies non-linearly with molecular size. Whereas a very small molecule, such as a dye of 400 mw, will approximately double its thickness in 10 minutes, transferrin (ca. 90,000 mw) will double in about three hours.

(8) Can "Disc Electrophoresis" be used as a medium for immuno-electrophoresis?

- Experiments to do immunological studies in the glass columns are yet to be carried out. If spread on slides, no advantage over agar is forseen.

(9) Can longer or fatter columns than the standard size (5 x 30 mm hard gel) be run?

- The standard size has been selected as that which provides the optimum combination between concentration and separation. The

shorter the column, the more tightly concentrated is each protein band. As you lengthen the column, the resolution (i.e. distance between bands) improves but the band itself becomes less sharp due to the diffusion of protein in the surrounding gel. A very long column would consist of broad, fuzzy bands very difficult to view or quantitate. Columns of larger diameter, when stained, tend to show a loss of sharpness in comparison to the standard size, more in the low molecular weight bands than in others. This is due to the longer time it takes for the fixative to diffuse to the center of a "fat" column. For batch separations (preparative) there is no important objection to "fatter" columns.

(10) Is a constant supply of current necessary?

- Unlike starch gel or starch block electrophoresis, in which the resistance of the sample is approximately constant throughout the run, resistance in these gel columns increases as the run proceeds. An unregulated supply will, therefore, pass less and less current through the columns as the run proceeds, will take unnecessarily long, and will therefore permit undesired diffusion of protein in the gel. If one has the time, patience, and motivation, one can use an unregulated supply and increase the current manually on a regular program throughout the run. Ideally this must be done in small, even increments and requires constant attention. A current-regulated supply provides the right amount of current to get the fastest run without overheating the columns, and permits you to equate distance of migration directly with time. Voltage regulation is of no use in this technique.

(11) Then why does your Model 1400 Research Power Source provide voltage control and regulation as well as current control and regulation?

- Because this unit is useful not only for "Disc Electrophoresis" but also as a general-purpose highly stable and versatile power source for starch, paper, and other electrophoretic techniques where voltage control may be desirable.

(12) And why do you provide the unregulated Model 150 Supply with the TRIAL KIT,

- Solely for the sake of economy. Purpose of the TRIAL KIT is to provide a means for you to try this powerful new technique on your own samples at minimum cost. The highest order of resolution is not called for under these circumstances. Loss of resolution to be expected because of the lack of current regulation will be no more than a doubling of band width in some fractions, and no observable loss in others. This will still be a startling improvement over other techniques. For optimum resolution, the Models 300 or 1400 are called for.

(13) What are the ionic strength and pH of the buffer, and can they be changed?

- The Standard buffer provided (specially developed for this system; do not try to use your regular buffers) has a pH of 8.3 and ionic strength of a little less than 0.01 in the bottle. When proteins are being separated, the pH in their environment is 9.4 and ionic strength 0.01. This buffer has been selected as that which offers optimum separation of most proteins in normal blood serum. pH and ionic strength can be changed to favor resolution of a particular protein, often resulting simultaneously in loss of resolution in another protein. A change in external buffer requires a corresponding change also in the gel. Instructions will provide details.

(14) What is the temperature rise during the run? Is cooling required?

- There is no temperature rise during the run, and cooling is not required. When using an unregulated supply (Model 150) there is a temperature drop during the run. When using a current regulated supply (Models 300 and 1400), temperature is constant.

(15) What dyes may be used in this system?

- The "tracking dye", which aids visual location of the moving "front" in the column, and which also marks the albumen during migration, may be any one of a number of negatively charged dyes which have greater mobility than albumen. Bromphenol blue is provided. The stain used after electrophoresis may be any one of the usual protein stains. Amido Schwartz is routinely provided. Several novel stains for special application are being developed.

(16) How do I quantitate the separated and dyed protein bands?

- If more accurate data than can be provided by a visual study is called for, the CANALCO Model E Densitometer is used. Because of the necessity to project a flat magnified image of a cylindrical sample, the special optics of the Model E are required. The usual spectrophotometer or densitometer created for other uses or other types of electrophoresis cannot be readily modified for this purpose.

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