CELLOGEL® RS
MULTIFRACTIONATIVE ELECTROPHORESIS (*) OF PROTEINS WITH A NEW ADVANCED TECHNIQUE ON SPECIAL CELLULOSE ACETATE GEL STRIPS

1. Myeloma serum: Multifractionation on Cellogel RS « wedge strip ». Applied quantity 2.5 μl
2. Normal serum: Multifractionation on Cellogel RS « wedge strip ». Applied quantity 2.5 μl
3. Normal serum: Multifractionation on Cellogel RS « rectangular strip » 1.5 μl/9 mm of 1:1 diluted sample
4. Unconcentrated urine (4.5 μl/9 mm) in a case of glomerular proteinuria
5. Unconcentrated urine (4.5 μl/9 mm) in a case of tubular proteinuria
5a. The above unconcentrated sample (30 μl as round spot) on « wedge strip ».
   Polaroid print through Chemetron Transilluminator.

CELLOGEL RS
offers many new properties

- Multifractionation without «molecular sieving» effect.
- Sensitivity 10-100 times superior to other electrophoretic techniques: with 0.25 μl serum you can see 16-23 fractions after 10 cm run!
- Direct electrophoresis of unconcentrated samples (urine**, cerebrospinal fluid etc.)
- Very simple technique: separation can be accomplished in 100 minutes with an old tank for paper electrophoresis (18 cm bridge) or in our inexpensive Chemetron tank (mod. 2000)


Cellogel is international trademark, registered in the States of the • Bureaux internationaux reüns pour la protection de la propriété industrielle • Genève.

CHEMETRON - 20129 MILANO (Italy)
Via Gustavo Modena, 24 - Tel. 720679 - 7380728
PROCEDURE FOR CELLOGEL RS (wedge or rectangular strips)
MULTIFRACTIONATIVE ELECTROPHORESIS (*)

1) Immerse Cellogel RS strips in the buffer 30 minutes before use. After blotting the strips between two filter paper sheets place them on the bridge (14.5 cm or 18 cm) of the electrophoretic tank.
2) Apply the sample on the penetrable surface at the cathode end. Use CHEMETRON applicators for serum protein (**) fractionation. Use a pipette dispenser for 20-50 or 100 μl application of un-concentrated samples as urine, cerebrospinal fluid etc. on "wedge strips".
3) Turn on the power supply and electrophoresis in the following conditions:

Buffer: Vetter pH 9
Bridge: 18 cm
Cellogel RS - wedge strips → Voltage and Time: 450 V - 100 min.
Cellogel RS 3x24 cm rectangular strips → Voltage and Time: 360 V - 130 min.
Bridge: 14.5 cm
Cellogel RS - wedge strips → Voltage and Time: 400 V - 60 min.
Cellogel RS = rectangular strips → Voltage and Time: 350 V - 80 min.

4) Stain with Lissamine Green solution for 5 min. and destain in 5% Acetic Acid.
5) After complete destaining only few fractions are visible, but all the microfractions become 100 times more intense after the following whitening treatment:
   a) Fix the strips in Methanol:Glycerol:Phenol (92:7:1 g)
   b) Place and roll the strip on a glass plate. Remove white strips after 20 minutes
   c) Examine against light of 60-100 W bulb at 2-3 cm distance. In this way colour of microfractions is photomultiplied.

SPECIAL PROCEDURES (more detailed instructions are included in packages of Cellogel RS)

RAPID MICROMETHOD FOR SERUM PROTEIN MULTIFRACTIONATION ON CELLOGEL RS 5.7 x 17 cm.

The present method requires a 45 minute migration. It consists in a prolonged microelectrophoresis with 7 cm run of the albumin. The obtained multifractionation is already sufficient for a clinical interpretation equivalent to what Laurell had expressed in his important work issued in 1972 (Scand. J. Clin. Lab. Invest., 29, suppl. 124, 71-82, 1972).

The herewith described method could become a definitive analytical technique for testing serum proteins. The strips after electrophoresis can be really used in two different ways:
1. Transparency treatment and densitometric reading of the usual 6-7 fractions.
2. Whitening treatment and observation (or photograph) of the microfractions (9-13), that become about 100 times more intense on white Cellogel RS strips.

Alternatively the strips which have undergone the second treatment can be made transparent giving again the usual fractionation if the second treatment is carried out before the first one.

(*) Detailed instructions are included in Cellogel RS packages.
(**) On "rectangular strips" apply 1.5 μl/μm of 1:1 diluted serum per strip. On "wedge strips" apply 2 times 1.5 μl/μm of undiluted serum.

METHOD

1. Immerse the Cellogel RS strips 5.7 x 17 cm in Vetter buffer diluted 40+60 H2O.
2. After a 20 minute impregnation remove the excess of liquid from the strips and lay them on the 11 cm bridge.
3. Apply four samples on each strip at 2 cm from the cathodic edge by means of a multiple micro-applicator (0.4 μl/4 mm). It is better to use diluted samples 2+1.
4. Connect the power supply at 300 V for 45 minutes exactly.
5. Stain the strips in a freshly prepared Amido-scharz solution.
6. Destain the strips in 3-4 baths until complete removal of the staining solution. The background of the strips is to be quite white.
7. Immerse the strips in methanol:glycerine:phenol (92:7:1 g) for one minute.
8. Lay the strips on a glass plate (the penetrable surface facing upward); remove the excess of liquid and peel away the strips after a 20 minute drying up at room temperature.
9. Observe the strips against a 100 W lamp in a dark room. Alternatively photograph the strips.

LIPOPROTEIN staining technique: After electrophoresis (short run) immerse strips for 3 hrs in the following freshly prepared solution:
40 mg Sudan Black B + 60 ml Ethanol + 70 ml 5% NaOH.
Wash in running water and preserve in water.
Separation of prelipoprotein is very sharp with Tris-Glycine Buffer.

GLYCOPROTEINS
After electrophoresis use the same staining technique as for normal Cellogel (Schiff reagent). Whitening process shows many glycoprotein fractions.

Normal serum proteins and corresponding glycoproteins (sample application: 0.5 μl/5 mm) on Cellogel RS 5 x 24 cm, after 15 cm run.

ESTERASIS
After electrophoresis, immerse the strips 1 second in an NaF solution. Lay on a glass plate. Incubate 30 min. at room temperature. Stain 1 second in Fast Blue RR solution. Store in water.

L D H Isozymes
Carry out electrophoresis with Cellogel RS « rectangular strips » on 14.5 cm bridge.
Buffer: Tris-Glycine pH 9.5 - Voltage 220 V - Time 75 min.
Start point: at the centre of the strips.
Sample quantity: 1-2 semimicroapplications (3 samples per strip).

Revelation: Apply 150 μl of MTT reagent with volumetric spreader Mod. DC/6. Incubate 20-30 min. at 37°. Fix in Formalin. Make whitening process on the following TRANSPARENCY PROCESS:
   a) immerse strips for 5 min. in Acetic Acid: H2O: Glycerol (30:69:1):
b) lay on a glass plate. Remove solution excess. Warm at 60-70° for 4-5 min. until complete transparence.

ISOENZYMES in general
The possibility of working with large sample quantity (30-50 μl) on "wedge strips" can resolve old problems in enzymoelektrophoresis. After electrophoresis use the same staining techniques as for normal Cellogel.

MULTIFRACTIONATION OF CASEIN (25-30 fractions)
Electrophoresis is accomplished with Cellogel RS "wedge strips" on 18 cm bridge - Voltage: 300 V Time: 2 hrs - Buffer: Veronal HCl-Urea.

MULTIFRACTIONATION OF MILK SERUM
Use the same conditions as for human serum proteins, but apply 6 μl sample on "wedge strips".

MULTIFRACTIONATION OF VEGETABLE PROTEINS
Proteins from corn, seeds, etc. are multilactionated in 90 min. at 300 V on 14.5 cm bridge. Buffer: Tris-Glycine pH 9.5.

MULTIFRACTIONATION OF MEAT AND FISH PROTEINS
The sample is homogenized and afterward it is squeezed with CHEMETRON SPUF Ultrafilter Syringe and pressed against Chemetron PR membrane. Electrophoresis is accomplished on 14.5 cm bridge in 90 min. at 300 V with Tris-Glycine buffer.

BIBIOGRAPHY ON CELLOGEL RS
URINE PROTEINS
L. Alessandrino - "Multifrazionamento proteico di urine non concentrate in Medicina del Lavoro Nucleare" - La Medicina del Lavoro - Vol. 63, no. 11-12 (1972).
L. Scarponi - "Electrophoresis of urine proteins on Cellogel and Cellogel RS" (in press).

<table>
<thead>
<tr>
<th>CHEMETRON MATERIALS FOR CELLOGEL RS MULTIFRACTIONATIVE ELECTROPHORESIS</th>
<th>Prices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power supply - 0-1000 V/0-150 mA</td>
<td></td>
</tr>
<tr>
<td>* Electrophoresis tank - Mod. 2000 - with extensible bridge 14.5-18 cm and 11 cm complete with cover and platinum electrodes</td>
<td></td>
</tr>
<tr>
<td>* Sample semimicroapplicator 1.5 μl/9 mm (code 134-136)</td>
<td></td>
</tr>
<tr>
<td>* Multiple microapplicator (4 samples of 0.4 μl each application) (code 161)</td>
<td></td>
</tr>
<tr>
<td>* Plastic guide for sample application (code 148)</td>
<td></td>
</tr>
<tr>
<td>Volumetric reagent spreader for enzymoelectrophoresis - Mod. DC/6</td>
<td></td>
</tr>
<tr>
<td>Chemetron SPUF Ultrafilter Syringe Press for squeezing homogenized materials, for squeezing segments of Cellogel Blocks (preparative electrophoresis) and for ultrafiltration on 30 mm ø membranes</td>
<td></td>
</tr>
<tr>
<td>Chemetron transiluminator for observation and photograph by Polaroid Land CU-5 - suitable for Cellogel RS and for 20 x 20 cm TLC plates</td>
<td></td>
</tr>
<tr>
<td>* Cellogel RS &quot;wedge strips&quot; 5 x 25 cm - 100 strips</td>
<td></td>
</tr>
<tr>
<td>* Cellogel RS 5 x 24 cm (rectangular strips) - 100 strips</td>
<td></td>
</tr>
<tr>
<td>* Cellogel RS 5.7 x 17 cm - 100 strips</td>
<td></td>
</tr>
<tr>
<td>* Tris-Glycine buffer (1 box of 10 packages for 10 l)</td>
<td></td>
</tr>
<tr>
<td>* VTter buffer (1 box of 10 packages for 40 lts)</td>
<td></td>
</tr>
<tr>
<td>PR membranes for ultrafiltration - pore size ø 0.45 μm - Thickness 300 μm, special for removal of mucilages from proteins - 1 package of 10 membranes ø 30 mm</td>
<td></td>
</tr>
</tbody>
</table>

* Recommended materials for the first trials.
Sample application in Chemetron tank with semimicro-applicator.

Micro Applicator for simultaneous application of 4 samples.

Semimicro (actual size)

Comparison of the same sera in the usual way (semimicro on normal Cellogel) and in the new way (micro on Cellogel RS).

Micro RS (actual size)

TECHNICAL ASSISTANCE:

Chemetron offers technical aid in case of difficulty, but it is necessary to send us a strip with defective results. We recommend in the first trials precise observance of the instructions: Lenght of run, buffer and sample quantity, if modified, can give you normal or distorted fractionation, but not multi-fractionation.

CHEMETRON PRODUCTS

Immunostrips®: for EID and RID.

Cellogel®: Cellulose acetate gel strips for usual electrophoresis and immunotechniques.

Cellogel Blocks: for preparative gel electrophoresis.

Cellogel RS: for multifractionative electrophoresis.

Electrophoresis equipments (Power supply - tanks).

Thin Layer Chromatography equipments and accessories.

(Automatic coater and linear sample applicator).

CHEMETRON DISTRIBUTORS: