2 D - Immuno-electrophoresis
TWO DIMENSIONAL IMMUNOELECTROPHORESIS ON CELLOGEL®

The cellulose acetate gel "Cellogel" is the ideal supporting medium for two dimensional immunoelectrophoresis.
The chief advantages of this technique are its speed*, simplicity** and the saving of antisera***. The equipment described below has been especially developed to perform two dimensional immunoelectrophoresis of plasma proteins, two dimensional immunoelectrophoresis of biologic liquids at low proteic concentration (unconcentrated urines, liquor, etc.), two dimensional immunoelectrophoresis with albumin standard for the planimetric quantitation of areas, tandem technique and the intermediate layer technique for peak identification. The method on Cellogel does not require a preventive carbamation.

* Reduced manipulation: Cellogel is ready to use.
** No refrigerated chamber is necessary.
*** A volume of 100 µL AS is used for 2 D–IEP of total SP.

EQUIPMENT

- Chemetron Power Pack 120-200 V (Code 10A01)
- Electrophoresis tank mod. 2/PAC-5 including two bridges, 8.5 cm and 11 cm (Code 11A03)
- Cellogel Variospeed (Code 13A34)
- Finnipette 5-50 µL (Code 13A12)
- Microcaps with Tygon tube of 0.5µL (Code 13A38-100) and 1µL (Code 13A39-100)
- Volumetric distributor DC/3 (Code 13A23)
- Volumetric distributor DC/6 (Code 13A24)
- Plastic template for two dimensional IEP (Code 13A27)
- Cellogel 14×14 cm, 200 µ, 10 sheets/kg (Code 01E06-10)
- Mylar sheets 180 µ, 18.3×14 cm (Code 13M06-100)
- Plastic container for staining-destaining (Code 13A21)

REAGENTS

- Buffer No. 7 TRIS-GLYCINE pH 9.5 – 10 packages (Code 02A01-10)
- Coomassie Brilliant Blue 250 R – 5 g (powder) (Code 03A05-P) or solution (Code 03C05-S)
- Destaining solution for Coomassie (Code 04A03-S)
- Bromophenol Blue 1 g (Code 03A07-P)
- Saline solution (0.9% NaCl in distilled water)
- Citric acid (Code 04A10-10)
- Clearing solution: 30 mL diacetonolcohol + 70 mL distilled water + 3 g TCA or Pedersen clearing powder (Code 06A07-P)

ANTISERA

- Pure Immunoglobulin antisera (Dakopatts, Denmark).
  For total SP use Rabbit Anti-Human serum Dako (Code A206) or Chemetron (Code 19S01-2)
REAGENT PREPARATION

TRIS-GLYCINE BUFFER  Dissolve the whole contents of one package in 1 liter of distilled water. Always use very fresh buffer and replace it after use. Clean and dry the electrophoresis tank perfectly before use.

STAINING SOLUTION  Coomassie BB/250R powder 0.5 g  
methanol 45 mL  
distilled water 45 mL  
acetic acid 10 mL  
The staining solution must be freshly prepared.

DESTAINING SOLUTION  Methanol 475 mL  
distilled water 475 mL  
acetic acid 50 mL

SALINE + BB  0.9% NaCl  
10 mL + 1 crystal Bromophenol Blue

Ecological preserving solution: Dissolve 1 pkg of Citric acid (25 g) in 1 liter of distilled water.

GENERAL COMMENTS

Present method and equipment is suitable to perform 1 to 4 two-Dimensional IEP with 2 Power Pack 120-200 V and 4 electrophoresis chambers. In fact for each sample 1 chamber is needed.

In the case of a large number of samples needing to be tested, ask for the price list of the Cellogel MULTIPOLAR SYSTEM with accessories for 2-Dimensional IEP. This compact system is able to perform not only 144 SPE in 1 hour and 16 to 20 simultaneous immunifications of monoclonal bands (16 to 20 patients × 5 tests) in 3 hours, but it is the first powerful tool on the market for 2-Dimensional IEP: 27 samples may be run at the same time (9 samples × one Multipolar chamber).

Note that Cellogel method is very sensitive, if compared with the agarose technique. More than 50 serum proteins may be identified on 0.50 μL of serum diluted 1:4 when new Dako rabbit AS Code A206 is used.

![Fig. 1](image1.png)  
Sheet and sample positioning

![Fig. 2](image2.png)  
Microcaps (0.5 μL) and plastic template
PROTOCOL FOR 2D-IEP
of serum proteins on Cellogel sheets 14×14 cm.

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<thead>
<tr>
<th>FIRST ELECTROPHORETIC MIGRATION</th>
<th>TIME</th>
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<tbody>
<tr>
<td>1. Equilibrate Cellogel sheets in the buffer for 15 min.</td>
<td>15 min.</td>
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<td>2. Fill 1 compartment of the chamber with buffer and level.</td>
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<td>3. Dilute samples 1:4, (1+3), with saline solution stained with a crystal of Bromophenol Blue.</td>
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<tr>
<td>4. Blot Cellogel between 2 filter paper sheets and place on the 8.5 cm bridge, penetrable dull surface uppermost. (Do not introduce bridge into the chamber before sample application).</td>
<td>1 min.</td>
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<tr>
<td>5. Using microcaps of 0.5 µL + Tygon tube apply diluted sample at the cathodic side 8 mm from the negative border and 35 mm from the edge of the sheet (see figure 1). Use the plastic template to help you.</td>
<td>1 min.</td>
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<tr>
<td>6. Introduce the bridge into the chamber. Observe if deposit is at negative (−) pole and cover the chamber.</td>
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Fig. 3
Loading of AS distributor

Fig. 4
Distribution of AS on Cellogel surface.
**ANTISERUM APPLICATION AND SECOND MIGRATION**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Time</th>
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<tbody>
<tr>
<td>1.</td>
<td>Disconnect power supply and remove the bridge with the sheet from the electrophoresis tank.</td>
<td>3 min.</td>
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<tr>
<td>2.</td>
<td>Dispense (see fig. 3) into the volumetric distributor DC/6 100 µL of the total AS (Dako A206). Add 50 µL of saline solution so that the optimum volume fullness (150 µL) for the distributor, is achieved.</td>
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<td>3.</td>
<td>Distribute the antiserum on the Cellogel surface at 0.5 cm from the Bromophenol Blue mark (4 cm from the Cellogel edge), moving the distributor back and forth until all the AS has been absorbed (and the surface loses its wet shine) (see fig. 4). Use the plastic template to help you.</td>
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<tr>
<td>4.</td>
<td>Remove the Cellogel sheet from the bridge and, after a 90° turn, place it on the bridge again.</td>
<td>1 min.</td>
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<tr>
<td>5.</td>
<td>Introduce the bridge into the electrophoresis tank with Bromophenol Blue mark at the negative (−) pole. Cover the tank.</td>
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<tr>
<td>6.</td>
<td>Electrophorese at 120 Volts overnight (12 to 16 hours).</td>
<td>overnight</td>
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<tr>
<td>7.</td>
<td>Disconnect power supply, remove sheet and wash it in saline solution: 6 baths of 15 min. each on a rotating shaker.</td>
<td>90 min.</td>
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<tr>
<td>8.</td>
<td>Stain in Coomassie staining solution for 15 to 30 min.</td>
<td>15 min.</td>
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<tr>
<td>9.</td>
<td>Destain in 3-4 baths of destaining solution (do not leave the sheet in the destaining solution longer than 1 hour).</td>
<td>20 min.</td>
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<tr>
<td>10.</td>
<td>Preserve in a large bath of 2.5% Citric acid</td>
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**VISUAL ESTIMATION OF 2 D-JEP**

Observe the wet sheet against a strong light and compare sample results with normal serum pattern. Store sheet in the bath of 2.5% Citric acid or in a sealed plastic transparent envelope. If a better intensification of weak precipitates is desired, use "optical enhancement method" described in the booklet N° 10 "Cellogel electrophoresis of unconcentrated urine and CSF proteins":

Destain the Cellogel sheet perfectly and immerse it in a solution of Glycerol:H₂O:Citric acid (8:92:5 g) for 3 min. Place on a glass plate and blot with a filter paper. Leave to stand in the open air until a dry white film is obtained (20 min.). Place on the window of the paper black mask (Code 13A40-10) and observe with the Transilluminator (Code 13A16) in a dark room. Store the dry membrane in a paper envelope. Cellogel sheet may be cleared in the usual way (Diacetonalcohol 30% in H₂O containing 3% TCA by warm procedure at 50°C) or better still using the following method that enhances the blue colour of Coomassie and sticks Cellogel onto Mylar:

1. Dissolve the contents of 1 pkg of Pedersen Powder in 200 mL H₂O.
2. Immerse Cellogel in the solution for 3 min.
3. Place on Mylar and introduce into an oven at 170-180°C for exactly 5 min. (no more).
2D-IEP OF BIOLOGICAL FLUIDS AT LOW PROTEIN CONCENTRATION (UNCONCENTRATED URINES, CSF, ETC.)

The same method as described for two dimensional Immunoelectrophoresis of serum proteins is used with the following variations of sample application and volume of antiserum:

- sample with total proteins less than 500mg/L: four deposits of 2µL (total 8µL). Antiserum: 30 µL + 120 µL saline
- sample with total proteins more than 500 mg/L: three deposits of 2 µL or less relating to total proteins. Antiserum: 40 to 50 µL + 110 to 100 µL saline.
- For CSF use Dako A208 antiserum (AS enriched with anti-prealbumin and anti-albumin). For Urine use Dako A211 antiserum.

2D-IEP: PEAK IDENTIFICATION INTERMEDIATE LAYER TECHNIQUE

Carry out the first migration of the sample according to the protocol at 200 V – 55 min. and afterwards:

- Load the volumetric distributor DC/3 with 5-10 or 15 µL of monovalent specific antiserum (or with a suitable mixture of monospecific antisera); then reload the DC/3 distributor until the optimum filling volume is achieved (50 µL total volume with saline).
- Use the perspex guide to coat the antiserum with the DC/3 distributor as already described.
- Load the DC/6 as usual, with Total AS (100 µL + 50 µL saline); distribute keeping the lateral edge of the spreader at a 2.5 cm distance from the mark left by Bromophenol during the first electrophoretic migration.
- Turn the sheet by 90° and perform second migration at 120 V, overnight.

TANDEM TECHNIQUE

Perform Two Dimensional Immunoelectrophoresis as in protocol with additional deposit of a pure antigen at a distance of 1 cm from the sample.
2D-IEP ON CELLOGEL:
STANDARDIZATION AND QUANTITATION.

In order to have the method standardized and a quantitative reference it is advisable to apply, in addition to the sample, a standard solution of pure human albumin 0.4%. The position of the deposit must be 2 cm ahead of the point of the sample deposit (third hole of the plastic template). The area of the peak of each protein is related to its quantity. This area can be measured by a planimeter.

REFERENCES


