HYDRAGEL 2 URINE PROFIL(E)
Ref. 4331

HYDRAGEL 4 URINE PROFIL(E)
Ref. 4332

Masque dynamique / Dynamic mask
INTENDED USE

The HYDRAGEL 2 URINE PROFIL(E) and HYDRAGEL 4 URINE PROFIL(E) kits are designed for identification of main urinary proteins to type renal failure (tubular, glomerular or mixed) and/or for qualitative detection and identification of polyclonal and monoclonal free light chains Kappa or Lambda (Bence Jones proteins), and for detection of polyclonal and monoclonal immunoglobulins G, A and M, in human urine or serum, by immunofixation electrophoresis on agarose gels with specific antisera.

The URINE PROFIL(E) test is performed in conjunction with the semi-automated HYDRASYS system to carry out all the steps needed to obtain gels ready for interpretation. Proteins are separated on alkaline buffered agarose gels (pH 9.1) and immunofixed with specific antisera against the proteins that represent various types of proteinuria.

Each agarose gel is intended to run:
- two samples in the HYDRAGEL 2 URINE PROFIL(E) kit,
- four samples in the HYDRAGEL 4 URINE PROFIL(E) kit.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

Detection and identification of proteinuria is helpful information in the diagnosis of renal failure. Proteinuria may result from many pathological conditions. Identification of the main proteins excreted into urine helps in pinpointing the type of the kidney damage (tubular, glomerular or mixed) and in diagnosis of the underlying pathology (Bence Jones proteins).

HYDRAGEL URINE PROFIL(E) is an immunofixation procedure using specific antisera. Immunofixation electrophoresis allows the proteins to be anchored in situ after electrophoresis, by forming insoluble complexes with corresponding antisera. The procedure allows a single-step characterization of the various proteinuria profiles and detection of Bence Jones proteins as a qualitative aid in the identification of monoclonal gammapathies.

To identify these abnormal bands, the technique of immunofixation is applied.

1. The sample is simultaneously electrophoresed in nine tracks on alkaline buffered agarose gel.
2. After the electrophoresis, one track (ELP) is fixed to serve as a reference showing electrophoretic pattern of the sample’s proteins. The remaining eight tracks are immunofixed with respective antisera.
3. The unprecipitated, soluble proteins are removed from the gel by blotting and washing. Precipitin of the antigen-antibody complex is trapped within the gel matrix.
4. The precipitated proteins are visualized by staining with acid violet. The excess of stain is removed with an acidic solution.

Proteins are immunofixed with specific antisera against:
- tubular proteins β2 microglobulin, Retinol Binding Protein (RBP) and α1 microglobulin,
- glomerular proteins (Albumin and α2 macroglobulin),
- heavy chains gamma, alpha and mu (with a trivalent antiserum),
- Kappa free and bound light chains,
- Lambda free and bound light chains,
- Kappa free light chain and,
- Lambda free light chain.

The immunoprecipitated bands are then compared with corresponding abnormal bands seen in the electrophoretic pattern of the sample and identified from the reaction, or lack of, with individual antisera.

This simple and fast technique gives a clear and easily interpretable picture.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 2 URINE PROFIL(E) AND HYDRAGEL 4 URINE PROFIL(E) KITS

<table>
<thead>
<tr>
<th>HYDRAGEL 2 URINE PROFIL(E) KIT</th>
<th>HYDRAGEL 4 URINE PROFIL(E) KIT</th>
<th>PN 4331</th>
<th>PN 4332</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
<td>10 gels</td>
<td></td>
</tr>
<tr>
<td>Buffered Strips (ready to use)</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
<td></td>
</tr>
<tr>
<td>Acid Violet Stain (stock solution)</td>
<td>1 vial, 75 mL</td>
<td>1 vial, 75 mL</td>
<td></td>
</tr>
<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10 (18 teeth)</td>
<td>2 packs of 10 each (18 teeth)</td>
<td></td>
</tr>
<tr>
<td>Antiseras segments (ready to use)</td>
<td>1 pack of 10 (18 wells)</td>
<td>1 pack of 10 (18 wells)</td>
<td></td>
</tr>
<tr>
<td>Filter Papers - Thin</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
<td></td>
</tr>
<tr>
<td>Filter Papers - Thick</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The fixative solution and the antisera are supplied separately from the kits (See REAGENTS REQUIRED BUT NOT SUPPLIED). FOR OPTIMAL RESULTS

All reagents from the same kit must be always used together and according to the package insert instructions.

PLEASE READ THE PACKAGE INSERT CAREFULLY.
1. AGAROSE GELS

Preparation
Agarose gels are ready to use. Each gel contains: agarose, 0.8 g/dL, tris-barbital buffer pH 9.1 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Agarose gels contain 0.31 % barbital and 0.34 % sodium barbital. Do not ingest! If ingested, consult physician immediately!

Use
Support medium for protein electrophoresis and immunofixation.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). The arrow on the front of the kit box must be pointing upwards). They are stable until the expiration date indicated on the kit package and the gel package labels. Prevent significant variations of temperature during storage, e.g., avoid storage close to a window or to a heat source.

DO NOT FREEZE.

Discard when:
(I) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(II) bacterial or mold growth is indicated,
(III) abnormal liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. BUFFERED STRIPS

Preparation
Buffered sponge strips are ready to use. Each contains: tris-barbital buffer pH 9.1 ± 0.3; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The buffer in the strips contains 0.92 % barbital, 1.03 % sodium barbital and 0.30 % sodium azide. Do not ingest! If ingested consult physician immediately! When disposing, prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide.

Use
Buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. The arrow on the front of the kit box must be pointing upwards). They are stable until the expiration date indicated on the kit package or buffered strips package label.

DO NOT FREEZE.

Discard buffered strips if the package is opened and the strips dry out.

3. ACID VIOLET STAIN

Preparation
Vial of the stock acid violet stain to be diluted up to 300 mL with distilled or deionized water. After dilution, the working stain solution contains: acid solution pH ≈ 2; acid violet, 0.2 g/dL; ethylene-glycol, 3.25 %; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Harmful if swallowed.

Use
For staining gels after protein electrophoresis and immunofixation.

IMPORTANT: The staining solution is designed to stain only 10 gels. Change the solution after 10 staining steps.

Storage, stability and signs of deterioration
Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicated on the kit package or stain vial labels. Working stain solution is stable for 6 months.

4. APPLICATORS

Use
Precut, single use applicators for sample application.

Storage
Store the applicators in a dry place at room temperature or refrigerated.

5. ANTISERA SEGMENTS

Use
Single use, colored segments for fixative solution and antisera application onto the gel for immunofixation with the dynamic mask.

WARNING: Segments loaded with antisera have to be handled with care as biological hazard.

6. FILTER PAPERS - THIN

Use
Single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

Storage
Store the thin filter papers in a dry place at room temperature or refrigerated.
7. FILTER PAPERS - THICK

Use
Single use, thick absorbent paper pads for blotting unprecipitated proteins off the gel after immunofixation step.

Storage
Store the thick filter papers in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED

1. ANTISERA AND FIXATIVE SOLUTION PACK
The antisera and fixative solution pack (SEBIA, PN 4335), GAM, K, L (anti-gamma, alpha, mu heavy chains, anti-Kappa free and bound light chains and anti-Lambda free and bound light chains), contains 3 antisera vials and 1 fixative solution vial, 1 mL each. They are specific for the immunofixation procedure with the dynamic mask.

IMPORTANT: In order to avoid any contamination between reagents, be careful to replace the cap on each corresponding vial after each use.

1.1. ANTISERA
Preparation
Ready to use. All antisera are mammalian, anti-human total immunoglobulins. For easy identification of antisera and as an aid in monitoring their application, the antisera are colored with distinct nonhazardous dyes that match the color of the vial label.

When antiserum exhibits a slight turbidity, leave the antiserum vial at room temperature for a minimum of 10 minutes. This should be sufficient to clear the solution; however, if turbidity remains, this should not affect in any way the immunological reaction. In case of insoluble precipitates, it is recommended to centrifuge antiserum for 5 minutes at 3000 rpm.

Use
For immunofixation of the electrophoresed proteins.

NOTE: The antisera are specific for the immunofixation procedure with the dynamic mask.

Antisera may originate from different animal species. Don't mix two different antisera vials, even with the same specificity, and ALWAYS change the tip of the pipette when changing antiserum vials.

Storage, stability and signs of deterioration
Store the antisera refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or antisera vial labels.

1.2. FIXATIVE SOLUTION
Preparation
Fixative solution is ready to use. It contains: acidic solution; additives, nonhazardous at concentrations used, necessary for optimum performance. For an easy identification and as an aid in monitoring its application, the fixative is colored with a nonhazardous dye.

Use
To fix electrophoretically separated proteins in the reference track (ELP).

NOTE: The fixative solution is specific for the procedure with the dynamic mask.

Storage, stability and signs of deterioration
Store fixative solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or fixative solution vial labels.

Fixative solution must be free of precipitate.

2. ANTISERA PACK FOR FREE LIGHT CHAINS
The antisera pack (SEBIA, PN 4336), anti-Kappa free light chains and anti-Lambda free light chains, for immunofixation contains 2 antisera vials, 1 mL each. They are specific for the immunofixation procedure with the dynamic mask. For easy identification of antisera and as an aid in monitoring their application, the antisera are colored with distinct nonhazardous dyes that match the color of the vial label.

Preparation, Use, Storage, stability and signs of deterioration: See previous paragraph 1.1.

IMPORTANT: In order to avoid any contamination between reagents, be careful to replace the cap on each corresponding vial after each use.

3. URINE PROFIL(E) ANTISERA PACK
The Urine Profil(e) antisera pack (SEBIA, PN 4338), anti-Tub. and anti-Alb/αM, for immunofixation, contains 2 antisera vials, 1 mL each (anti-tubular proteins and anti-glomerular proteins). They are specific for the immunofixation procedure with the dynamic mask. For easy identification of antisera and as an aid in monitoring their application, the antisera are colored with distinct nonhazardous dyes that match the color of the vial label.

Preparation, Use, Storage, stability and signs of deterioration: See previous paragraph 1.1.

IMPORTANT: In order to avoid any contamination between reagents, be careful to replace the cap on each corresponding vial after each use.

NOTE: During transportation, the antisera can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

4. DESTAINING SOLUTION
Preparation
Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials, 100 mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container. After dilution, the working destaining solution contains: citric acid, 0.5 g/L.

Use
For destaining, that is removal of excess and background stain from the gels.

For rinsing of the staining compartment after wash step.

To neutralize the acidity of the destaining solution, pour 15 mL of a 50 % solution of sodium hydroxide into the empty waste container.

Storage, stability and signs of deterioration
Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. DO NOT FREEZE. Working destaining solution is stable for one week at room temperature in a closed bottle. Do not add any sodium azide.
Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
To prevent microbial proliferation in the working destaining solution to be stored more than one week, add 5 µL/dL of ProClin 300. Then, the solution is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

5. HYDRASYS WASH SOLUTION
Preparation
Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3 ; sodium azide.
WARNING: The stock wash solution contains 0.625 % sodium azide. Do not ingest ! If ingested, consult physician immediately ! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acids, lead or copper. Always flush with a large quantity of water when disposing.

Use
The HYDRASYS wash solution is designed to wash unprecipitated proteins from gels. It also serves for cleaning of the HYDRASYS's Staining Compartment. Use periodically, e.g., if the instrument is used daily, wash the staining compartment weekly.
See the package insert for directions to use.

Storage, stability and signs of deterioration
Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

6. SALINE
Preparation
Make 0.15 M (0.9 g/dL) NaCl solution in distilled or deionized water
Use
To dilute samples if necessary.

Storage, stability and signs of deterioration
Store at room temperature or refrigerated. Discard after 3 months or if it changes its appearance, e.g., becomes cloudy due to microbial contamination. For longer storage periods, add sodium azide, 0.1 g/dL.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED
1. HYDRASYS System SEBIA, PN 1210 or PN 1211.
2. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216 or HYDRAPLUS 2 SEBIA, PN 1217, for an alternative way of loading the sample applicators or antisera segments.
3. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.
4. Container Kit supplied with HYDRASYS.
5. Template guide Bar SEBIA supplied with HYDRASYS.
6. Dynamic mask, SEBIA, PN 1255.
7. Accessory kit for URINE PROFIL(E), Dynamic mask, SEBIA, PN 1274. It contains: one colored reference guide and one length reducing device, specific for URINE PROFIL(E) procedure.
8. Pipettes: 6 µL, 8 µL, 10 µL and 200 µL.

SAMPLES FOR ANALYSIS
Sample collection and storage
• The analysis is generally carried out on fresh urine. If needed, store urines at 2 to 8 °C for up to one week. For longer storage periods, keep urines frozen. Freezing with HEPES 0.1 M (pH 6.75) and / or sodium azide, 0.02 g/dL improves the storage stability. Frozen urines are stable for at least one month. Thawed samples can give slight application marks due to protein denaturation.
IMPORTANT: Avoid boric acid as preservative.
• If analyzing sera, they must be collected according to established procedures used in clinical laboratory testing. If needed, store sera at 2 to 8 °C for up to one week. For longer storage periods, freeze the samples. Frozen sera are stable at least for one month. Thawed samples may give slight application marks due to protein or lipoprotein denaturation.

Sample preparation
Urinés
Analysis is performed on unconcentrated urine. The detection level of Bence Jones protein is generally within 1 - 5 mg/dL. Concentrate urine if required by the procedures established in the laboratory or if a higher sensitivity is needed. A 20 - 100 fold concentration is generally sufficient. The typification of the kidney damage with Anti-Tub. and Anti-Alb/αM antisera must be performed on unconcentrated urines.
NOTE : Diffusion of urine samples into the applicator tips may be hindered when the urine (neat or concentrated) is turbid. It is recommended to remove the particulates by centrifugation (e.g., 10 minutes at 3,000 rpm) or filtration (e.g., 0.45 µm syringe filter).
Sera
In addition to urine, the patient’s serum may also be tested for Bence Jones protein and complete monoclonal immunoglobulins.

Dilute the serum in saline or in diluent for immunofixation previously diluted 1/4 (1 part diluent, 3 parts distilled or deionized water): 1/10 for ELP, GAM, K and L tracks (1 part serum, 9 parts diluted diluent or saline) and 1/3 (1 part serum, 2 parts diluted diluent or saline) for Kf and Lf tracks.

NOTE : If total immunoglobulin level is < 0.5 g/dL, it is recommended to use lower dilutions of the serum sample in saline or in the diluent for immunofixation previously diluted 1/4. For example, dilute 1/5 the serum for ELP, GAM, K and L tracks (1 part serum, 4 parts diluted diluent or saline) and 1/2 (1 part serum, 1 part diluted diluent or saline) for Kf and Lf tracks.

For Ig D and/or Ig E analysis, use the HYDRAGEL 2 URINE PROFIL(E) or HYDRAGEL 4 URINE PROFIL(E) techniques with the Standard mask, SEBIA, PN 4831 and 4832, and apply the same dilutions as for Kappa and Lambda free and bound light chains.

For serum analysis, immunofixation in tracks Tub and Alb/αM is not required.

WARNINGS:
- Avoid plasma samples. Fibrinogen gives a band in the reference track close to the application point which might be taken for a monoclonal immunoglobulin.
- Some urines contain high concentration of salts. This may cause gel deformation during migration and consequently, distortion of the migration profiles. If such distortion makes interpretation ambiguous, the urine should be dialyzed to eliminate the salts.
- Due to bacterial contamination, the immunoglobulin (paraprotein) present in urine may undergo proteolysis. This would lead to a positive reaction with anti free light chain antisera. In such case, a serum paraprotein eliminated in urine may show a monoclonal band with the trivalent antisera, and with one of the anti free and bound antisera and with the corresponding anti free light chain antisera.
- Polymerization of Bence Jones proteins generally lowers the sensitivity of detection with the anti free light chains antisera as the polymerization may block the epitopes that normally react with anti free light chain antisera. When no detection is achieved and polymerized Bence Jones proteins are suspected, depolymerize prior to electrophoresis: mix 100 µL urine with 5 µL beta-mercaptoethanol diluted 1:10 with saline and apply.

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, incubation with fixative solution and antisera, drying, washing, staining, destaining and final drying.

The manual steps include handling samples and gels, application of fixative and antisera and setting up the instrument for operation.

IMPORTANT: Adjust the dynamic mask position for perfect alignment between electrophoretic profiles and wells of the mask (See the package insert for directions to use for dynamic mask adjustment).

I. MIGRATION SET UP
1. Switch on HYDRASYS instrument.
2. Place one applicator for two samples analysis on HYDRAGEL URINE PROFIL(E) 2/4 or two applicators for 4 samples analysis, on a flat surface with the well numbers in the right-side-up position (Fig. 1):
   - Load each applicator within 2 minutes.

<table>
<thead>
<tr>
<th>TRACK</th>
<th>SAMPLE No. 1 OR 3</th>
<th>SAMPLE No. 2 OR 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELP</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Tub</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Alb αM</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>GAM</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>DE</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>K</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>L</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Kf</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Lf</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

Mark all tracks that are not used to prevent interpreting them as negative results.
- Place the applicator(s) into the wet storage chamber with the teeth up [handle it (them) by the plastic tooth protection frame].
- Let the samples diffuse into the teeth for 5 minutes after the last sample application.
- See wet chamber package insert for further details.

3. Open the lid of the Migration Module and raise the electrode and applicator carriers.
   WARNING: Never close the lid while the carriers are raised !
4. Select ‘2/4 BJ-UP SM/DM’ migration program for HYDRAGEL 2 and 4 URINE PROFIL(E) procedures, from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package : handle them by the plastic ends. Engage the punched ends of the strip’s plastic backing to the pins on the electrode carrier : the strip’s plastic backing must face the carrier (Fig. 2).
6. Unpack the HYDRAGEL plate.
   - Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately.
   - Pool 200 µL distilled or deionized water on the lower third of the frame printed on the temperature control plate of the migration module.
   - Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 3).
   - Bend the gel and ease it down onto the water pool (Fig. 3). Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.
7. Lower both carriers down. In this position the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.

8. Remove the applicator(s) from the wet chamber. Handle it (them) by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - Place the applicator(s) on the carrier:
     - With HYDRAGEL URINE PROFIL(E) 2/4 (2 samples), into position No. 3.
     - With HYDRAGEL URINE PROFIL(E) 2/4 (4 samples), into positions No. 3 and 9.

   **IMPORTANT:** The numbers printed on the applicator(s) must face the operator (Fig. 4).

   To improve reproducibility of sample application, always push the applicators to the left side of the carrier.

9. Close the lid of the migration module.

10. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard.

   **IMPORTANT:** Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

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**MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS**

- The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out under 20 W constant until 42 Vh accumulated (for about 9 minutes), at 20 °C controlled by Peltier effect.
- The electrode carrier rises to disconnect the electrodes.
- An audible beep signals that the migration module lid unlocks.

The following message is displayed on the screen: "H AS".

**NOTE:** The migration module lid remains locked during all migration steps.

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**II. IMMUNOFIXATION SET UP**

The dynamic mask is made of a colored reference guide for reagent application, an antisera segment, a segment holder, a dynamic mask guide and a specific length reducing device, contained in the Accessory kit for URINE PROFIL(E), Dynamic mask, SEBIA, PN 1274 (Fig. 5).

During the migration, assemble the dynamic mask as follows:

1. Place the dynamic mask guide on a flat surface.

   **IMPORTANT:** For two samples analysis, it is necessary to position one length reducing device at the top of the dynamic mask guide.

2. Set up an antisera segment on the segment holder (Fig. 6):
   - Tilt the antisera segment at a 45° angle and position it against the plastic springs of the segment holder.
   - Pull apart the two elements and pivot the segment to fix it into the notches of the segment holder.

   **WARNING:** Be sure the segment is correctly positioned on the holder: the pins at ends of the segment must be blocked into the notches of the holder.

3. Set up the holder with the segment on the dynamic mask guide (Fig. 7) (containing the length reducing device placed for two samples analysis). Then, put the colored reference guide for reagent application, corresponding to the assay being run, on the segment holder in front of the segment wells (Fig. 8).

4. Apply reagents as follows:
   - 18 wells antisera segment for HYDRAGEL URINE PROFIL(E) 2/4:
     - 6 µL per well for 2 samples analysis,
     - 8 µL per well for 4 samples analysis.

<table>
<thead>
<tr>
<th>TROUGH</th>
<th>REAGENT</th>
<th>COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELP</td>
<td>fixative solution</td>
<td>yellow</td>
</tr>
<tr>
<td>Tub</td>
<td>anti-Tub. antiserum</td>
<td>azure (sky blue)</td>
</tr>
<tr>
<td>Alb αM</td>
<td>anti-Alb/αM antiserum</td>
<td>green</td>
</tr>
<tr>
<td>GAM</td>
<td>trivalent antiserum</td>
<td>violet</td>
</tr>
<tr>
<td>K</td>
<td>anti-Kappa light chain (free &amp; bound) antiserum</td>
<td>light green</td>
</tr>
<tr>
<td>L</td>
<td>anti-Lambda light chain (free &amp; bound) antiserum</td>
<td>light blue</td>
</tr>
<tr>
<td>Kl</td>
<td>anti-Kappa free light chain antiserum</td>
<td>orange</td>
</tr>
<tr>
<td>Lf</td>
<td>anti-Lambda free light chain antiserum</td>
<td>red</td>
</tr>
</tbody>
</table>

   **NOTES:**
   - Reagents are colored and the colors are shown on the colored reference guide to facilitate correct antisera pipetting.
   - Please refer to each printed plastic support of the gels for reagent application in antisera segments supplied for each procedure.
   - Aspirate reagents avoiding any air bubbles in the pipette tip.
   - Apply the reagents (Fig. 9):
     - Hold pipette at an angle and rest its tip lightly at the side of the well, without touching the bottom of the well.
     - Inject the drop of reagent into the well.

5. Remove the colored reference guide.

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**III. IMMUNOFIXATION**

1. Open the migration module lid.

2. Remove the sample applicator(s) and discard.

3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Wipe electrodes with soft wet tissue.
   - Leave the gel in place in the migration module.

4. Set up the dynamic mask for reagent application as follows (Fig. 10):
   - Position the mask guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the dynamic mask by the tab and position it into the guide with the notches aligned with the marks.
   - Lower the dynamic mask onto the plate of HYDRASYS.
5. Place the segment holder at the lowest point on the mask guide, facing the operator. Hold the segment holder by the handle situated on its right and press on the central pressure point such that the antisera segment contacts the gel.

**WARNING**: Do not press again on the pressure point at any time during the immunofixation step as this may result in cross contamination of the reagents.

Release the pressure; then, reagents will spread under each track (Fig. 11).

6. Immediately, using the segment holder handle, move the segment slowly but steadily up and down the entire length of the gel to apply the reagents. Application should take approximately 5 seconds (Fig. 12).

**WARNING**: During this step, hold the mask only by the segment holder handle. Avoid touching the guide.

7. Remove the guide and the dynamic mask.
   - Remove the segment holder using its handle.
   - Remove the antisera segment from the holder and discard.

**WARNING**: Segments loaded with antisera have to be handled with care as biological hazard.

**NOTE**: Residual reagent may remain in the wells after application. This should have no effect on test results.

8. Close the lid of the migration module

9. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard.

**NOTE**: The migration module lid remains locked during incubation.

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**IMMUNOFIXATION - DESCRIPTION OF THE AUTOMATED STEPS**

- Incubation at 20 °C for 10 minutes (controlled by Peltier effect).
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: " ■ PAP."

**NOTE**: The migration module lid remains locked during incubation.

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**IV. BLOWING OF THE GEL**

1. Open the migration module lid.
2. Apply a thick filter paper on the gel: line up the filter paper edge with the gel edge (incline it at a 45° angle) and ease it down onto the gel.

**IMPORTANT**: Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.

3. Close the lid of the migration module.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

**NOTE**: The migration module lid remains locked during incubation.

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**BLOWING - DESCRIPTION OF THE AUTOMATED STEPS**

- Blowing at 40 °C controlled by Peltier effect, for 3 minutes.
- An audible signal (beep) rings.

The following message is displayed on the screen: " ■ PAP."

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**V. DRYING OF THE GEL**

1. Open the migration module lid.
2. Remove the filter paper and leave the gel in place.
3. Close the lid.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

**NOTE**: The migration module lid remains locked during incubation.

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**DRYING - DESCRIPTION OF THE AUTOMATED STEPS**

- Drying at 50 °C controlled by Peltier effect, for 6 minutes.
- A beep signals that the cover unlocks. The plate temperature remains at 50 °C until the lid is opened. “Migration temp maintained” is displayed on the screen.

**NOTE**: The migration module lid remains locked during the drying step.

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**VI. GEL PROCESSING SET UP**

1. Open the lid.
2. Remove the dried gel film for further processing.
3. Open the gel holder. Lay it flat and position the dried gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder (Fig. 13).
4. Place the gel holder into the Gel Processing / Staining Module.

**IMPORTANT**: Before starting the gel processing / staining program, check the following:
- the wash solution container contains at least 400 mL of wash solution;
- the staining container is filled with 300 mL of staining solution;
- the destaining container contains at least 1 liter of destaining solution;
- the waste container is empty.

For reagent line connection: Refer to the information displayed on the screen of the instrument (select key: REAGENT LINES).

**IMPORTANT**: Do not forget to block up the unused lines.

5. Select “IF ACID VIOLET” staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

During staining, destaining and drying steps, the compartment remains locked.

After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).
NOTES:
- Temperature of the migration plate keeps decreasing since the lid has been opened until it reaches 20 °C (in less than 5 minutes). Then a new migration run may start.
- Return the sample applicator and electrode carriers back in place.
- Wipe the temperature control plate with a soft wet tissue.

VII. GEL PROCESSING COMPLETION
1. Remove the gel holder from the compartment, open it and remove the dried gel.
2. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.

NOTE: The lengths of electrophoretic migrations may be slightly different with gels containing 2 analysis rows, without any adverse effects on performance.

RESULTS

Interpretation

Physiological proteinuria
It is weak, generally lower than 120 mg / 24 h and there is no significant difference between male and female. In cases of physiological proteinuria, albumin fraction appears with low intensity (with possibly traces of transferrin on the ELP reference track).

Tubular proteinuria
The tubular proteinuria is characterized by the presence of one or the three proteins detected by the Anti-Tub. antiserum (α-1 microglobulin, β-2 microglobulin and RBP, retinol binding protein).

Glomerular proteinuria
The glomerular proteinuria is characterized by the presence of albumin detected by the Anti-Alb antiserum; the presence of albumin alone is characteristic of selective glomerular proteinuria. If the gamma zone or a monoclonal component is detected with the corresponding GAM track, the type of proteinuria will be classified non-selective glomerular proteinuria. Positive reaction with the Anti-αM antiserum indicates presence of high molecular weight proteins, due to blood traces in some cases (post-renal proteinuria). It is recommended to perform the analysis on a new sample without any blood.

Mixed proteinuria
The mixed proteinuria is characterized by the presence of both tubular and glomerular proteins, e.g., proteins detected by Anti-Tub. and Anti-Alb/αM, anti-Ig GAM and Anti-Kappa and Lambda light chains.

Bence Jones proteinuria or free light chains in serum
Presence of a Bence Jones protein in urine or free light chain in serum is characterized by:
- a monoclonal band detected with one of the anti-free and bound light chains Kappa or Lambda antisera, (K or L tracks),
- the same monoclonal band detected with the corresponding anti-free light chains (Kappa or Lambda) antisera, (Kf or Lf tracks),
- no reaction with the trivalent antiserum (GAM track).

When Bence Jones protein is lower than 5 mg/dL, a single band may be detected with one of the anti-free and bound light chain antiserum, without any reaction with the corresponding anti-free light chain antiserum. In such a case, no reaction with anti-free light chain and anti-Ig GAM antiserum (without Ig D and Ig E serum monoclonal component) indicate the presence of Bence Jones protein as there is no heavy chain corresponding to the detected free light chain. A Bence Jones protein must be detected as one monoclonal band. The presence of a diffuse zone in Kappa and / or Lambda tracks indicate polyclonal free light chains which are not Bence Jones proteins.

Limitations
The use of antisera other than those specific for the immunofixation procedure with the dynamic mask may affect the results. Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some monoclonal components may not be detected with this method.

Troubleshooting
Call Technical Service of the supplier when the test fails to perform while the instructions for the preparation and storage of materials, and for the procedure were carefully followed.
Kit reagent Safety Data Sheets and information on waste product elimination are also available from the Technical Service of the supplier.

PERFORMANCE DATA

Standard materials, sample preparation and procedures were used. All electrophoregrams were evaluated visually.

Reproducibility within gel and lot to lot
Reproducibility within gel was demonstrated on three different pathological samples: One urine sample with Bence Jones protein (free Lambda light chain), one urine with glomerular proteinuria and one urine with tubular proteinuria. Each sample was run 4 times on 2 lots of HYDRAGEL URINE PROFIL(E) 2/4 gels using the acid violet staining procedure.

With HYDRAGEL 4 URINE PROFIL(E) procedure, the urinary proteins were correctly identified in each sample and on all gels, there were no false positives and no differences were observed among the repetitive assays. For each urine sample tested, identical results were obtained within lot and lot-to-lot experiments showing typical pattern and specific urinary proteins as expected for each sample type.
Reproducibility between gels and lot to lot

Reproducibility between gels was demonstrated on four different pathological samples with respectively, tubular proteinuria, glomerular proteinuria, tubular proteinuria associated with free Lambda light chain and Kappa Bence Jones protein.

These samples were run on 10 HYDRAGEL URINE PROFIL(E) 2/4 gels from 3 different lots, using the acid violet staining procedure.

With HYDRAGEL 4 URINE PROFIL(E) procedure, identical results were obtained within gel, gel-to-gel and lot-to-lot experiments. The urinary proteins were correctly identified in each sample on all gels, there were no false positives and false negatives, and no differences were observed among the repeats. For each urine sample tested, typical pattern and specific urinary proteins were detected as expected for each sample type.

Accuracy

Fifty one (51) urine samples, including two physiological urines, were run using the HYDRAGEL 4 URINE PROFIL(E) kit and two other commercially available agarose gel systems for urine analysis, using molecular sieving and immunofixation procedures, respectively.

Eleven (11) serum samples with Bence Jones proteins (monoclonal free Kappa or Lambda light chains) and two normal sera, were run using the HYDRAGEL 4 URINE PROFIL(E) kit and another commercially available agarose gel immunofixation system.

For all the analyzed urine and serum samples, the results obtained with the individual procedures were in agreement and the same protein bands were detected on all pathological samples with each system.

Sensitivity

Serial dilutions were prepared with typical samples exhibiting different urinary proteins and analyzed with HYDRAGEL URINE PROFIL(E) procedures with specific antisera.

The minimal detection limit was about 0.6 mg/dL for tubular proteins and 0.3 mg/dL for glomerular proteins. Due to the structure and the type of the free light chain and their polymerization, the detection limit is generally around 2 mg/dL with anti-Kappa and anti-Lambda (free and bound light chain) antisera and around 5 mg/dL with anti-Kappa and anti-Lambda free light chain antisera. But for some Bence Jones proteins, the detection limit determined by serial dilution of pathological samples, was obtained about 0.3 mg/dL with anti-free and bound light chains antisera and about 1.2 mg/dL with anti-free light chains antisera.

BIBLIOGRAPHY

(1) Brouet J.C. Orientation diagnostiquée en cas d’anomalies des immunoglobulines plasmatiques (Ig E exclues). La Revue du Praticien, n° 9, 21/03/91, p. 782 à 785.
SCHÉMAS / FIGURES

Figure 1

Figure 2

Figure 3

Figure 4
Figure 5

Repère couleurs
Colored Reference Guide

Barrette antisérums
Antisera Segment

Support barrette
Segment Holder

Réducteur de course
Length Reducing Device

Guide du masque dynamique
Dynamic Mask Guide