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HYDRAGEL 6 IF Penta

Ref. 4841

HYDRAGEL 12 IF Penta

Ref. 4842

Ref. 4884*

Masque standard / Standard mask

IVD

CE

2012/03

INTENDED USE

The HYDRAGEL 6 IF Penta and HYDRAGEL 12 IF Penta kits are designed for the detection in large majority of monoclonal proteins in human serum by immunofixation electrophoresis on alkaline buffered (pH 9.2) agarose gels. They are used in conjunction with the semi-automated HYDRASYS system to perform all the steps needed to obtain gels ready for interpretation. Serum proteins are electrophoresed and immunofixed by a pentavalent antiserum anti-gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and anti-kappa and lambda (free and bound) light chains. After immunofixation, the precipitated proteins are stained with amidoblack. The excess of stain is removed with an acidic solution.

Each agarose gel in the HYDRAGEL 6 IF Penta and HYDRAGEL 12 IF Penta kits is intended to run six or twelve samples, respectively.

For *In Vitro* Diagnostic Use.

NOTE : In this instruction sheet, the name "HYDRASYS" is used for both semi-automated HYDRASYS and HYDRASYS 2 instruments.

PRINCIPLE OF THE TEST

Abnormal bands in the electrophoretic pattern of serum proteins, generally with a gamma-mobility, but also alpha- and beta-mobility (these might be hidden by regular proteins of the corresponding zones), are always suspect of being monoclonal proteins and therefore an indication of gammopathies. To identify these abnormal bands, the technique of immunofixation is applied.
Immunofixation electrophoresis is a simple technique that allows a protein to be anchored *in situ* after electrophoresis, by forming an insoluble complex with its antibody. It is performed in four stages:

1. Separation of proteins by electrophoresis on agarose gel.
2. Fixation and immunoprecipitation of the electrophoresed proteins: fixative solution and antiserum are overlaid directly onto the gel surface over the appropriate electrophoretic migration tracks. The fixative solution and antiserum diffuse into the gel, precipitating all the proteins and the corresponding antigens, respectively.
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. The immunoprecipitated bands are then compared with the corresponding abnormal bands seen in the electrophoretic pattern of serum sample.

To detect the suspect monoclonal component(s), the samples are simultaneously electrophoresed in two tracks. After electrophoresis, the ELP track serves as a reference showing electrophoretic pattern of the sample's proteins. The immunofixation (Penta) track reveals monoclonal components that reacted with the pentavalent antiserum.
This simple and fast technique gives a clear and easily interpretable picture.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 6 IF Penta AND HYDRAGEL 12 IF Penta KITS

WARNING : See the safety data sheets.

ITEMS	PN 4841	PN 4842	PN 4884*
Agarose Gels (ready to use)	10 gels	10 gels	80 gels
Buffered Strips (ready to use)	10 packs of 2 each	10 packs of 2 each	80 packs of 2 each
Staining solution diluent (stock solution)	1 vial, 60 mL	1 vial, 60 mL	8 vials, 60 mL
Amidoblack Stain (stock solution)	1 vial, 20 mL	1 vial, 20 mL	8 vials, 20 mL
Fixative Solution (ready to use)			3 vials, 14.4 mL
Mammalian anti-Ig human immunoglobulins (gamma - alpha - mu - kappa - lambda) : pentavalent antiserum (ready to use)			3 vials, 8.0 mL
Applicators (ready to use)	1 pack of 10	2 packs of 10 each	16 packs of 10 each
Filter Papers-Thin	1 pack of 10	1 pack of 10	8 packs of 10 each
Paper combs	1 pack of 10	2 packs of 10 each	16 packs of 10 each
Filter Papers-Thick	1 pack of 10	1 pack of 10	8 packs of 10 each

** HYDRAGEL 12 IF Penta MAXI-KIT*

NOTE : The fixative solution and the Pentavalent antiserum are supplied separately from the kits except for MAXI-KIT (See REAGENTS REQUIRED BUT NOT SUPPLIED).
During transportation, the MAXI-KIT can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

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 ; buffer solution pH 9.2 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for protein electrophoresis and immunofixation.

Storage, stability and signs of deterioration
Store gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards). Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.
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 : buffer solution pH 9.1 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Buffered sponge 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. STAINING SOLUTION DILUENT

Preparation
The stock staining solution diluent must be used as described in paragraph " AMIDOBLACK STAIN ".
It contains an acidic solution pH ≈ 2.

Use
For the preparation of the amidoblack staining solution.

Storage, stability and signs of deterioration
Store the stock staining solution diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or staining solution diluent vial labels. DO NOT FREEZE.
Do not add any sodium azide.

4. AMIDOBLACK STAIN

Preparation
The amidoblack concentrated stain is a visquous solution which may gelify. The integrity of the stock staining solution is not altered by the increase in viscosity or solidification.
In all cases, to obtain a perfect reconstitution of the stain, we advise you to respect the following procedure:
1. Add 15 mL of stain diluent to the concentrated amidoblack vial.
2. Close carefully the vial.
3. Shake very vigorously the vial during approximately 5 seconds.
4. Pour this solution in the container for staining solution processing.
5. Repeat this step twice, three times if necessary.
6. Pour the remaining diluent in the container and complete the volume to 300 mL with distilled or deionized water.
7. Mix contents of stain cubitainer well for 5 to 10 minutes.

The staining solution is ready to use.

NOTE : An incomplete reconstitution of the stain will lead to an under-evaluation of albumin fraction (low percentage or white hole inside the fraction).

After dilution, the working staining solution contains : acidic solution pH ≈ 2 ; amidoblack ; ethylene-glycol ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
For staining gels with electrophoretic protein separations.

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 staining solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock staining solution is stable until the expiration date indicated on the kit package or staining vial labels.
Working staining solution is stable for 1 month. Its stability may be extended for 3 months if the working solution is refrigerated. The closed container must be stored refrigerated immediately after each use.
Do not store the working staining solution close to a heat source.

5. FIXATIVE SOLUTION (with PN 4884)

Preparation
Fixative solution is ready to use. It contains : acidic solution pH ≈ 2 ; additives, nonhazardous at concentrations used, necessary for optimum performance. For easy identification and as an aid in monitoring its application, the fixative is colored with a nonhazardous yellow dye that matches the color of the vial label.

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

NOTE : The fixative solution is specific for the immunofixation procedure with the 6 and 12 IF Penta masks.

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

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

6. PENTAVALENT ANTISERUM (with PN 4884)

Preparation

Pentavalent antiserum is ready to use. Pentavalent antiserum is a mixture of mammalian total anti-Ig human immunoglobulins. For easy identification and as an aid in monitoring its application, the antiserum is colored with a nonhazardous yellow-orange dye that matches 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 Pentavalent antiserum is specific for the immunofixation procedure with the 6 and 12 IF Penta masks.

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.

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

Storage, stability and signs of deterioration

Store the Pentavalent antiserum refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or antiserum vial labels.

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

7. APPLICATORS

Use

Precut, single use applicators for sample application.

Storage

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

8. 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.

9. FILTER PAPER COMBS

Use

Precut, single use, thick absorbent paper combs for blotting excess of fixative solution and antiserum off the gel surface after immunofixation step.

10. 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

WARNING : See the safety data sheets.

1. FIXATIVE SOLUTION

Preparation

Fixative solution for immunofixation (SEBIA, PN 4799, 1 vial, 2.5 mL) is ready to use. It contains : acidic solution pH ≈ 2 ; additives, nonhazardous at concentrations used, necessary for optimum performance. **The fixative solution is specific for the immunofixation procedure with the 6 and 12 IF Penta masks, SEBIA.**
For an easy identification and as an aid in monitoring its application, the fixative is colored with a non hazardous yellow dye that matches the color of the vial label.
Use, Storage, stability and signs of deterioration : See previous paragraph 5.

2. PENTAVALENT ANTISERUM

The Pentavalent antiserum vial (SEBIA, PN 4616, 1 mL or PN 4738, 8 mL), contains a mixture of mammalian total anti-Ig human immunoglobulins. **It is specific for the immunofixation procedure with the 6 and 12 IF Penta masks, SEBIA.**
For easy identification and as an aid in monitoring its application, the antiserum is colored with a nonhazardous yellow-orange dye that matches the color of the vial label.
Preparation, Use, Storage, stability and signs of deterioration : See previous paragraph 6.

3. 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 an acidic solution pH ≈ 2.

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. 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 diluted destaining solution to be stored more than one week, add 5 µL/dL of ProClin 300.
Working destaining solution added with ProClin 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.

4. 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 : buffer solution pH 8.7 ± 0.5.

Use
The HYDRASYS wash solution is designed to wash unprecipitated proteins from gels. It also serves for cleaning of the HYDRASYS 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.

5. FLUIDIL

Preparation
Fluidil (SEBIA, PN 4587, 1 vial, 5 mL) is ready to use.

Use
To dilute viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel.

Storage, stability and signs of deterioration
Store at room temperature. It is stable until the expiration date indicated on the Fluidil vial label.
Fluidil must be free of precipitate.

NOTES :

The assays that were performed for the validation of reagents demonstrated that, for the different solutions and using an adapted equipment for the reconstitution volume, a variation of ± 5 % on the final volume has no adverse effect on the analysis.
The distilled or deionized water used to reconstitute solutions, must be free of bacterial proliferation and mold (use a 0.22 µm filter) and have a resistivity higher than 10 Megohms x cm.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. HYDRASYS System SEBIA: HYDRASYS 2 SCAN PN 1200, HYDRASYS 2 PN 1201, HYDRASYS 2 SCAN FOCUSING PN 1202, HYDRASYS 2 FOCUSING PN 1203, HYDRASYS PN 1210 or PN 1211 or HYDRASYS FOCUSING PN 1212.
2. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216, HYDRAPLUS 2 SEBIA, PN 1217 or ASSIST SEBIA, PN 1218, for an alternative way of loading the sample applicators.
3. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.
4. Container Kit supplied with HYDRASYS.
5 Template guide Bar SEBIA, supplied with HYDRASYS.
6. Accessory Kit for HYDRASYS IF, SEBIA, PN 1260.
7. Pipettes: 10 µL and 200 µL.

8. For optional quantitative analysis of the protein fractions on the "fixative" track : densitometer / scanner capable of scanning 82 x 102 mm gel plates : HYRYS SEBIA, GELSCAN SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer's instructions for operation and calibration procedures.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh serum samples are recommended for analysis. Sera 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 samples are stable for at least one month.
Thawed samples may give slight application marks due to protein or lipoprotein denaturation.
Frozen serum samples with sodium azide, 0.02 g/dL improves the storage stability.

Sample preparation
Use undiluted serum samples.
After storage at 2 to 8 °C or freezing, some sera (particularly those containing a cryoglobulin or cryogel) may become viscous or develop turbidity. Such sera might present application problems due to hindered diffusion through the sample applicator teeth. In such case add 25 µL Fluidil to 75 µL of serum and vortex for 15 seconds. Then follow the standard procedure.
Some monoclonal proteins can polymerize resulting in a "monoclonal fraction" appearing on all immunofixed tracks. In this case (i) prepare 1 % beta-mercaptoethanol (BME, or 2-mercaptoethanol, 2ME) in Fluidil, (ii) add 25 µL of this reducing solution to 75 µL neat serum, (iii) vortex and wait at least 15 minutes minimum (maximum 30 minutes) and then follow the standard procedure.

Sample to avoid
Avoid plasma samples: fibrinogen gives a band in the reference track close to the application point that might be taken for a monoclonal immunoglobulin.
Avoid hemolyzed samples.

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 antiserum, drying, staining, destaining and final drying. The manual steps include handling samples and gels, application of fixative and antiserum and setting up the instrument for operation.
READ CAREFULLY HYDRASYS / HYDRASYS 2 INSTRUCTION MANUAL.

I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator for 6 samples analysis on HYDRAGEL IF Penta 6/12 or two applicators for 12 samples analysis, on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL neat sample in each well. Load each applicator within 2 minutes.

	WELLS	
SAMPLES	**ELP**	**PENTA**
1, 7	2	3
2, 8	4	5
3, 9	6	7
4, 10	9	10
5, 11	11	12
6, 12	13	14

   NOTE: The wells no. 1, 8 and 15 are not used in this test ; they may be marked with a fiber tip pen to avoid filling them with samples by mistake.
   - Place each applicator into the wet storage chamber with the teeth up [handle it by the plastic tooth protection frame].
     See wet chamber package insert for further details.
   - Let the samples diffuse into the teeth for 5 minutes after the last sample application. For later use (up to 8 hours), keep the entire chamber under refrigeration.
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 "6/12 PENTA SM/DM" migration program 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.
   WARNING: Do not leave the filter paper for a too long contact with the gel to avoid its dehydration.
   - Pool 200 µL of 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 each applicator from the wet chamber. Handle it by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - For 6 samples analysis on HYDRAGEL IF Penta 6/12, place the applicator into position No 6 on the carrier.
   - For 12 samples analysis, place the two applicators each into position No 3 and 9.

   To improve reproducibility of sample application, always position the applicators on the left side of the carrier.
   IMPORTANT: The numbers printed on the applicator must face the operator (Fig. 4).
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.

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 at 20 °C controlled by Peltier effect, until 42 Vh accumulated (for about 9 minutes).
- 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: "⇩ AS" / "⇩ ANTISERA".

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

II. IMMUNOFIXATION SET UP

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 the electrodes with soft wet tissue.
   - Leave the gel in place in the migration module.
4. Set up the reagent application template as follows (Fig. 5):
   - Position the application template guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the template by the flap and insert it into the template guide (the notches against the marks).
   - Lower the template onto the gel.

5. Apply reagents as follows:

TROUGH	VOLUME	REAGENT	COLOR
ELP	35 µL	fixative solution	yellow
Penta	25 µL	pentavalent antiserum	yellow - orange

NOTE: To avoid mix-up, reagent colors are shown both on the vial labels and the application template wells.
- *Take the reagents without trapping any air bubbles in the pipette tip.*
- *Apply the reagents (Fig. 6):*
  - *Hold pipette vertically and rest its tip lightly at the bottom of the well.*
  - *Inject reagent so it spreads through the trough without trapping any bubbles.*

6. Close the lid of the migration module.
7. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard. A message "[INCUBATION]" appears on the screen.

IMMUNOFIXATION - DESCRIPTION OF THE AUTOMATED STEPS

- Incubation at 20 °C for 5 minutes (controlled by Peltier effect).
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: "⇧ AS (SM)" / "⇧ ANTISERA (SM)".

NOTE: The migration module lid remains locked during incubation.

III. REMOVAL OF THE EXCESS OF REAGENTS

1. Open the migration module lid.
2. Remove the excess of reagents with the filter paper combs (one comb for HYDRAGEL 6 IF Penta, two combs for HYDRAGEL 12 IF Penta) (Fig. 7):
   - Insert each comb at a 30° angle into the slots at the lower end of the template troughs so that the teeth touch the vertical side away from the operator.
   - Allow the teeth to contact delicately the liquid by tilting each comb to a 45° angle enabling the teeth to wick off the liquid (Fig. 8).

   IMPORTANT: Each comb must stay inclined (45°). If it is straight up, it could damage the gel.
3. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

REAGENT REMOVAL - DESCRIPTION OF THE AUTOMATED STEPS

- The reagents are allowed to wick off the troughs for 15 seconds at 20 °C (controlled by Peltier effect).
- An audible beep rings. The following message is displayed on the screen: "⇩ PAP." / "⇩ THICK FILTER PAPER".

IV. BLOTTING OF THE GEL

1. Remove the comb(s).
2. Check that the reagents are well absorbed as indicated by:
   - the absence of reagents on the gel.
   - the full lengths of the teeth are stained.

   If the reagent absorption is incomplete, insert the same filter paper comb again (in the same position) and repeat manually the removal procedure.
3. Grasp the reagent application template by the flap, lift it and remove it.
4. Apply a thick filter paper on the gel:
   - line up the filter paper edge with the gel edge,
   - ease it down onto the gel (incline it to a 45° angle).

   IMPORTANT: Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.
5. Close the lid of the migration module.
6. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).
7. Clean the application template with a small brush (e.g., toothbrush). DO NOT USE ALCOHOL OR SOLVENTS. Ensure the template is completely dry before re-use ; remove water droplets from the wells by tapping it on soft paper.

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS

- Blotting at 40 °C controlled by Peltier effect, for 3 minutes.
- An audible signal (beep) rings. The following message is displayed on the screen: "⇧ PAP." / "⇧ THICK FILTER PAPER".

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).

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.

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

VI. GEL PROCESSING SET UP

1. Open the lid.
2. Remove the dried gel 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. 9).

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 AMIDO" 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.
3. Scan using a densitometer / scanner by selecting the appropriate scanning program (optional).

NOTE : The lengths of electrophoretic migrations may be slightly different with gels containing 2 or 3 analysis rows, without any adverse effects on performance.

QUALITY CONTROL

It is recommended to run an assayed control serum (such as IT / IF Control, SEBIA PN 4788) after each change of lot of a reagent.
For optional quantitative analysis of the protein fractions on the "fixative" track : It is advised to include an assayed control serum (Normal Control Serum, SEBIA, PN 4785) into each run of samples.

** US customers : Follow federal, state and local guidelines for quality control.*

RESULTS

The pentavalent antiserum detects:
- G, A, M immunoglobulins, kappa and lambda each,
- free kappa and lambda light chains,
- kappa and lambda light chains bound to epsilon or delta heavy chains.

Interpretation

A **normal serum** shows a slightly stained zone, corresponding to the polyclonal immunoglobulins (G, A, M, kappa and lambda), without any sharp band.
A **hypergammaglobulinemia** is characterized by a heavily stained, diffused zone, without any restricted bands.
A **gammopathy** is characterized by one or more focused band(s).

Special cases
- A slight monoclonal gammopathy can be hidden in a normal electrophoretic profile (e.g., beta-mobility dysglobulin). This band will be detected with the pentavalent antiserum.
- When a monoclonal type band is observed on serum electrophoresis (ELP track) but fails to be confirmed by immunofixation, fibrinogen (plasma sample) should be the prime suspect.
- When a monoclonal type band is observed on all immunofixation tracks, cryoglobulin or polymerized Ig M should be suspected. Depolymerize with a reducing agent and repeat the procedure (see "Samples for analysis").

Any monoclonal-like fraction has to be identified by further investigation on HYDRAGEL Immunofixation kits with the Standard mask :
- HYDRAGEL 1 IF SEBIA, PN 4801, HYDRAGEL 2 IF SEBIA, PN 4802, HYDRAGEL 4 IF SEBIA, PN 4804 or 4808, or HYDRAGEL 9 IF SEBIA, PN 4809,
- HYDRAGEL 1 BENCE JONES SEBIA, PN 4821, HYDRAGEL 2 BENCE JONES SEBIA, PN 4822 or HYDRAGEL 4 BENCE JONES SEBIA, PN 4824.

Values (for optional quantitative analysis of the protein fractions on the "fixative" track)

Densitometer scanning of stained electrophoregrams on the "fixative" track of the gel yields relative concentrations (percentages) of individual protein zones.
Normal values (mean ± 2 SD) for individual major electrophoretic serum protein zones on HYDRAGEL IF Penta 6/12 gels have been established from a healthy population of 101 adults (men and women).

The protein quantification in UV on CAPILLARYS gives similar values to nephelometric procedure (especially for albumin). SEBIA proposes a HYDRAGEL – CAPILLARYS/NEPHELOMETRIC Equivalency of values obtained on HYDRAGEL after calibration of scanning systems.

	Without HYDRAGEL CAPILLARYS/NEPHELOMETRIC Equivalency Values	With HYDRAGEL CAPILLARYS/NEPHELOMETRIC Equivalency Values
FRACTION	**HYRYS - GELSCAN - PHORESIS**	**HYRYS - GELSCAN - PHORESIS**
Albumin	59.8 – 70.9 %	53.8 – 63.8 %
Alpha-1 globulins	1.4 – 3.2 %	1.6 – 4.0 %
Alpha-2 globulins	7.5 – 12.3 %	8.6 – 15.3 %
Beta globulins	8.0 – 12.7 %	9.2 – 15.8 %
Gamma globulins	8.5 – 15.7 %	9.8 – 19.5 %

It is recommended each laboratory establishes its own normal values.

Limitations

The use of antisera other than those specific for the immunofixation procedure with the standard 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.
This technique, as every immunofixation technique, depends totally on the sensitivity and the specificity of antisera. Antisera are biological products with critical manufacturing control. SEBIA has developed some advanced tests dedicated to the different antisera batches ; nevertheless, some monoclonal components might not be detected.
The immunofixation technique using the Pentavalent antiserum is a screening technique to be imperatively completed by more definite analyses, such as immunofixation techniques carried out for the whole range of the specific antisera.

Troubleshooting

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

PERFORMANCE DATA

Qualitative analysis

Reproducibility and specificity

Reproductibility was demonstrated on two pathological serum samples with high and low level of monoclonal component, respectively, one serum with an increased alpha1-alpha2 zone and one normal serum. Each sample was run on 3 lots of HYDRAGEL 12 IF Penta kit. All tested samples gave identical results for the 3 lots showing patterns typical for the type of sample tested. Immunofixation yielded no monoclonal band with the serum showing an inflammatory pattern and with the normal serum.

Accuracy

Thirty different samples (29 pathological sera and one normal serum sample) were run using the HYDRAGEL 12 IF Penta kit, HYDRAGEL IF kit, and another commercially available agarose gel immunofixation system. Identical bands were detected on all pathological samples with each system or procedure.

Sensitivity

Serial dilutions were prepared with 3 pathological serum samples all exhibiting monoclonal components.
The results are summarized below.

MONOCLONAL COMPONENT			DETECTION LIMIT (g/L)	
SAMPLE No.	**TYPE**	**CONC. (g/L)**	**HYDRAGEL 12 IF Penta**	**COMPARATIVE TEST**
1	gamma, kappa	18	0.25	0.25
2	alpha, lambda	7.3	0.12	0.5
3	mu, kappa	9	0.5	0.5

Quantitative analysis of the serum protein fractions on the "fixative" track

The following results obtained after quantitative analysis of the serum proteins on the "fixative" track using the HYDRAGEL 6 & 12 IF Penta Standard mask procedure indicate a very good reproducibility within gel, between runs and lot-to-lot with a mean CV % of about 2.1 % for each protein fraction, after densitometric scanning with HYRYS.
The results obtained with the PHORESIS scanner are equivalent (with a mean CV % of about 2.9 %).

Reproducibility within run

Two different serum samples were each run in 12 tracks on HYDRAGEL IF Penta 6/12 gels from 2 lots with the Standard mask and the specific fixative solution. The means (%), SD and CV % (n = 12) were calculated for each serum sample, each zone and each lot.

The following table shows the values for each protein fraction of the 2 tested samples analyzed on the 2 lots of gels:

FRACTION	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Serum A : lot No. 1 / lot No. 2					
MEAN (%)	50.1 / 50.3	4.2 / 4.4	13.7 / 13.6	16.1 / 15.8	16.0 / 16.0
SD	0.40 / 0.40	0.15 / 0.18	0.16 / 0.25	0.17 / 0.15	0.20 / 0.38
CV (%)	0.8 / 0.8	3.7 / 4.2	1.1 / 1.9	1.1 / 1.0	1.3 / 2.3
Serum B : lot No. 1 / lot No. 2					
MEAN (%)	63.9 / 64.1	2.3 / 2.3	10.2 / 10.2	10.8 / 10.6	12.8 / 12.8
SD	0.33 / 0.54	0.14 / 0.11	0.20 / 0.19	0.27 / 0.22	0.21 / 0.29
CV (%)	0.5 / 0.8	5.9 / 4.7	2.0 / 1.9	2.5 / 2.1	1.7 / 2.3

Reproducibility between runs and between and lot-to-lot

Twelve different serum samples were run 10 times on HYDRAGEL IF Penta 6/12 from 3 lots with the Standard mask and the specific fixative solution. The means (%), SD and CV % (n = 10) were calculated for each serum sample and each zone.
The following table shows, for each fraction, the limit values for the 12 tested samples and a mean CV % calculated from the pooled CV's for all samples (n = 12):

FRACTION	MEAN (%)	SD	CV (%)	MEAN CV (%)
Albumin	49.9 - 70.9	0.27 - 0.51	0.5 - 0.9	0.6
Alpha-1	1.7 - 5.0	0.05 - 0.25	1.9 - 6.4	3.9
Alpha-2	8.1 - 15.0	0.08 - 0.30	0.8 - 2.7	1.6
Beta	8.7 - 15.9	0.10 - 0.45	1.0 - 3.7	1.7
Gamma	7.8 - 20.4	0.17 - 0.45	1.0 - 3.6	2.3

Accuracy

Pathological and normal serum samples (n = 96) were run using the HYDRAGEL 12 IF Penta Standard mask procedure with quantitative analysis of the serum proteins on the " fixative " track and another commercially available agarose gel system for protein quantification. The correlation parameters calculated for individual zones from the pooled data for HYDRAGEL 12 IF Penta vs. the comparative gel system (y = HYDRAGEL 12 IF Penta) were:

Fraction	Correlation coefficient	y-intercept	Slope	Range of % values HYDRAGEL 12 IF Penta
Albumin	0.991	0.063	0.978	29.6 - 72.7
Alpha-1	0.944	0.104	1.058	1.1 - 7.3
Alpha-2	0.986	-0.218	1.036	4.8 - 21.7
Beta	0.990	0.710	0.986	5.0 - 43.3
Gamma	0.998	0.610	0.984	2.7 - 57.5

Linearity

The quantitative analysis of serum proteins on the "fixative" track with the HYDRAGEL 12 IF Penta Standard mask test and the specific fixative solution was determined to be linear to at least 4.1 g/dL albumin and 2.3 g/dL gammaglobulins.

Sensitivity

Serial dilutions of one serum sample with a monoclonal protein at 4.4 g/dL were electrophoresed using the HYDRAGEL 12 IF Penta Standard mask procedure. The highest dilution with a discernible monoclonal band on the "fixative" track corresponded to a concentration of about 34 mg/dL of the monoclonal component.

NOTE: According to the position of the monoclonal component and polyclonal background in the gamma zone, the detection limit may vary.

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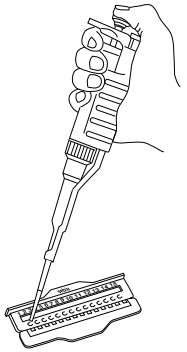
SCHÉMAS / FIGURES

Figure 1



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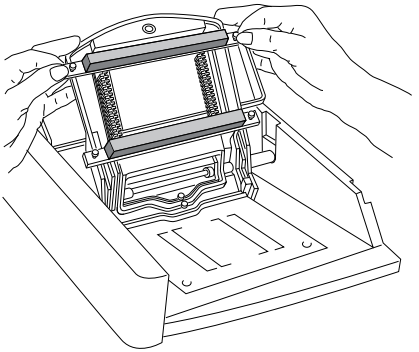

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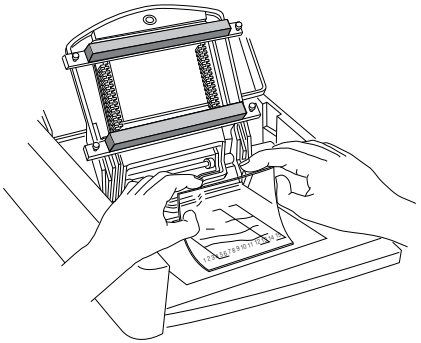

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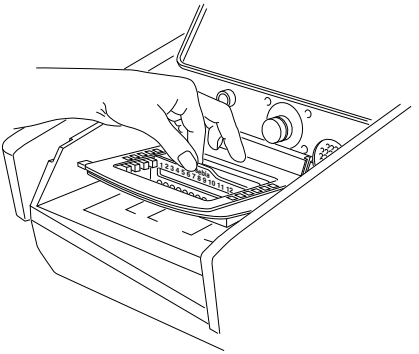

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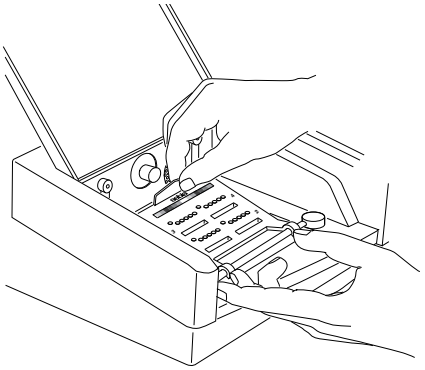

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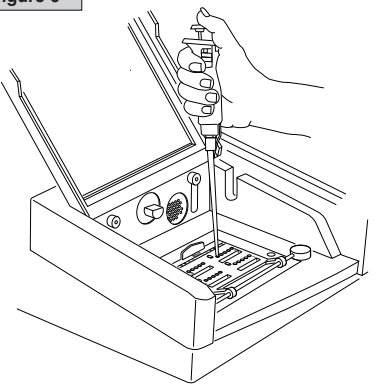
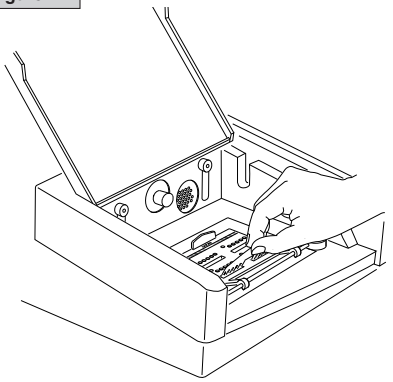


SCHÉMAS / FIGURES

Figure 7



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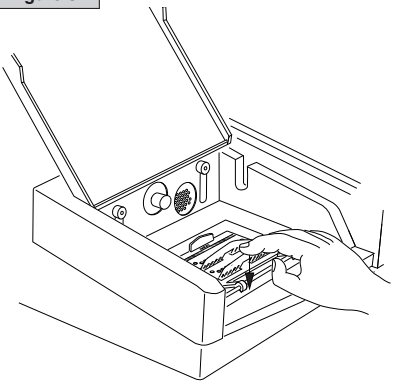
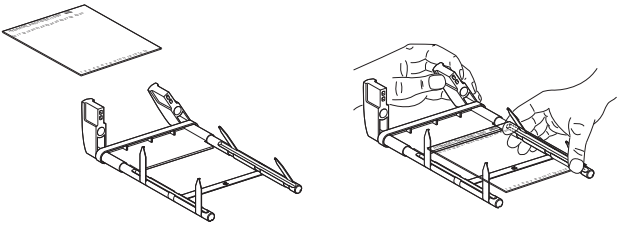


Figure 9

HYDRASYS



HYDRASYS 2