

**sebia**

**HYDRAGEL 7 PROTEIN(E)**

Ref. 4100

**HYDRAGEL 15 PROTEIN(E)**

Ref. 4120

**HYDRAGEL 30 PROTEIN(E)**

Ref. 4140

IVD

CE

R<sub>x</sub>only

2020/07

## INTENDED USE

The HYDRAGEL 7 PROTEIN(E) and HYDRAGEL PROTEIN(E) 15/30 gels are intended for separation of human serum proteins in human serum and urine by electrophoresis on alkaline buffered (pH 9.1) agarose gels. By design, the normal human serum proteins separate into five major fractions. The kits are used in conjunction with the semi-automated HYDRASYS instrument. The separated proteins are stained with amidoblack. The electrophoregrams are evaluated visually for pattern abnormalities. Densitometry provides accurate relative quantification of individual zones.

Each agarose gel is intended to run:

- 7 samples in the HYDRAGEL 7 PROTEIN(E) kit,
- 15 samples in the HYDRAGEL 15 PROTEIN(E) kit,
- 30 samples in the HYDRAGEL 30 PROTEIN(E) kit.

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<sup>1-15</sup>

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening of serum and some other fluids for protein abnormalities. It is based on the principles of zone electrophoresis performed on a suitable support medium. Agarose has been developed into a versatile and effective support medium. For routine diagnostic applications, serum proteins separate into five major fractions, primarily according to their charge at a given pH: albumin, alpha-1 globulins, alpha-2 globulins, beta globulins and gamma globulins. Each zone contains one or more serum proteins. The urine protein patterns resemble those of serum. However, the relative intensities of the fractions or their presence may vary greatly depending on the filtration capability of the kidney.

## REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7, 15 AND 30 PROTEIN(E) KITS

**WARNING :** See the safety data sheets.

ITEMS	PN 4100	PN 4120	PN 4140
Agarose Gels (ready to use)	10 gels	10 gels	10 gels
Buffered Strips (ready to use)	10 packs of 2 each	10 packs of 2 each	10 packs of 2 each
Staining solution diluent (stock solution)	1 vial, 60 mL	1 vial, 60 mL	1 vial, 60 mL
Amidoblack Stain (stock solution)	1 vial, 20 mL	1 vial, 20 mL	1 vial, 20 mL
Applicators (ready to use)	1 pack of 10 (7 teeth)	1 pack of 10 (15 teeth)	2 packs of 10 (15 teeth)
Filter Papers	1 pack of 10	1 pack of 10	1 pack of 10

**FOR OPTIMAL MANAGEMENT OF TRACEABILITY :** All reagents from the same kit must be used together.

**TO OBTAIN THE EXPECTED PERFORMANCES :** The package insert instructions must be observed.

### 1. AGAROSE GELS

#### Preparation

Agarose gels are ready to use. Each gel contains : agarose ; buffer solution pH 9.1 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

#### Use

Support medium for protein electrophoresis.

#### 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). 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 quantity of liquid 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 9.2 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

#### 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 (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 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 "AMIDOBBLACK STAIN".

It contains an acidic solution pH = 2.

#### Use

For the preparation of the amidobblack staining solution.

#### Storage, stability and signs of deterioration

Store the stock staining solution diluent at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). 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. AMIDOBBLACK STAIN

#### Preparation

The amidobblack 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 amidobblack 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 ; amidobblack ; 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 (15 to 30 °C) or refrigerated (2 to 8 °C) 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 (2 to 8 °C). The closed container must be stored refrigerated (2 to 8 °C) immediately after each use.

Do not store the working staining solution close to a heat source.

### 5. APPLICATORS

#### Use

Precut, single use applicators for sample application.

#### Storage

Store the applicators in a dry place at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C).

### 6. FILTER PAPERS

#### Use

Precut, 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 (15 to 30 °C) or refrigerated (2 to 8 °C).

### REAGENTS REQUIRED BUT NOT SUPPLIED

**WARNING :** See the safety data sheets.

### 1. 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 % (W/W) solution of sodium hydroxide, commercially available, into the empty waste container ( $\approx$  19 M NaOH).

**Storage, stability and signs of deterioration**

Store the stock destaining solution at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). 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 (15 to 30 °C) 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 or CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL).

See the *CLEAN PROTECT* package insert for directions to use.

Working destaining solution added with ProClin or CLEAN PROTECT is stable in a closed bottle at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C) until the expiration date indicated on the kit package or destaining solution vial labels.

**2. 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**

It 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 (15 to 30 °C) or refrigerated (2 to 8 °C). 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.

**3. FLUIDIL****Preparation**

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

**Use**

To dilute samples with hindered diffusion through the sample applicator teeth (for example, viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel, samples with polymerized Ig M, ...) or giving low intensity electrophoretic pattern.

**Storage, stability and signs of deterioration**

Store at room temperature (15 to 30 °C). 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 filter ≤ 0.45 µm) and have a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.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. Pipettes: 10 µL and 200 µL.
6. Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 mm gel plates : HYRYS SEBIA, GELSCAN SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer's instructions for operation and calibration procedures.
7. Gel holder for half gels, SEBIA, PN 1278.

**SAMPLES FOR ANALYSIS****Sample collection and storage**

Fresh serum samples are recommended for analysis. Serum and urine must be collected according to established procedures used in clinical laboratory testing. Refrigerate samples (2 to 8 °C) as soon as possible after collection for up to one week. For longer storage periods, keep samples frozen (stable at least one month).

Freezing serum samples with sodium azide, 0.02 g/dL improves the storage stability.

Freezing urine samples with HEPES 0.1 M (pH 6.75) and sodium azide, 0.02 g/dL improve the storage stability.

**IMPORTANT:** Avoid boric acid as preservative.

Thawed samples can give slight application marks due to protein or lipoprotein denaturation. Storage at 2 to 8 °C and freezing cause anodic shift of beta-lipoproteins from beta-zone to alpha-2 or alpha-1 zones ; the older the serum, the greater the shift.

## Sample preparation

### 1. Sera

Use undiluted serum samples.

Treatment of serum samples with Fluidil :

The treatment of serum samples with Fluidil must be applied in the following cases :

- Serum samples with hindered diffusion through the sample applicator teeth, for example, viscous or turbid sera after storage at 2 to 8 °C or after freezing (particularly those containing cryoglobulin or cryogel).
- Serum samples with polymerized Ig M.
- Serum samples giving low intensity electrophoretic pattern.

In such cases, add 25 µL Fluidil to 75 µL serum and vortex for 15 seconds. Then follow the standard procedure.

### 2. Concentrated urines

Analysis is performed on samples previously concentrated to a total protein concentration of about 1.5 - 2.0 g/dL (with an adapted device).

**IMPORTANT:** Some urines have a salt content. This can cause a gel deformation during migration and consequently, distortion of the migration profiles. If such a distortion makes interpretation inaccurate, the urine should be dialyzed to remove the salts.

*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 (Follow the usual recommendations on pre-analytical phase applied to urine samples analysis) or filtration (e.g., 0.45 µm syringe filter).*

## Sample to avoid

- Avoid hemolysed serum samples. Hemolysis increases alpha-2 and beta-zones.
- Avoid plasma samples. Fibrinogen gives a band close to the application point which might be taken for a monoclonal immunoglobulin and would offset percentage of corresponding zone.
- Avoid aged, improperly stored urine samples where enzymatic degradation of proteins might occur.

*NOTE : Collection tubes and centrifugation parameters for biological samples are described in the available documentation on pre-analytical phase for bio-medical analysis (data provided by the tube manufacturers, guides and recommendations on biological sample collection...). Without any indication in the instructions for use on the type of tube to use or on the centrifugation, please refer to this documentation and for the dimensions of tube to use, refer to the SEBIA document "Characteristics of tubes to use according to the instrument". The pre-analytical phase must be performed according to the state of art, the different recommendations, including those provided by the tube manufacturers, and applicable regulations.*

## 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, drying, staining, destaining and final drying. The manual steps include handling samples and gels, 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 HYDRAGEL 7 PROTEIN(E) (7 samples) and HYDRAGEL PROTEIN(E) 15/30 (15 samples), or two applicators for HYDRAGEL PROTEIN(E) 15/30 (30 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 1).
  - Apply 10 µL of neat serum sample or concentrated urine in each well. Load each applicator within 2 minutes.
  - 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. For later use (up to 8 hours), keep the entire chamber under refrigeration.

*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 "7 PROTEIN(E)" migration program for HYDRAGEL 7 PROTEIN(E) or "15/30 PROTEIN(E)" migration program for HYDRAGEL PROTEIN(E) 15/30 from the instrument menu.
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 120 µL of distilled or deionized water for HYDRAGEL 7 PROTEIN(E), or 200 µL for HYDRAGEL PROTEIN(E) 15/30, 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.
    - **Examine the applicator :** The application of a sample in the well of an applicator leads to a change in appearance of the corresponding tooth, which passes from white to translucent or more or less colored, according to the type of sample (serum, blood, urine, diluent...). Before placing an applicator on the applicator carrier, check that all teeth of the applicator are soaked with sample by examining its back side. A white tooth indicates a failure in the application or in the diffusion of the sample (defective applicator membrane, sample not applied, turbid or viscous...). In the absence of diffusion, use a new applicator and apply the samples again (treated or not according to the procedure).
    - Snap off the applicator teeth's protection frame.
    - For 7 and 15 samples analysis, place the applicator into position No 6 on the carrier.
    - For 30 samples analysis, place the two applicators each into position No 3 and 9.

**IMPORTANT:** The numbers printed on the applicator(s) 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.

**IMPORTANT:** Make sure that the ventilation air inlet 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 10 W constant for HYDRAGEL 7 PROTEIN(E) or 20 W constant for HYDRAGEL PROTEIN(E) 15/30, at 20 °C controlled by Peltier effect, until 33 Vh have accumulated (for about 7 minutes).
- The electrode carrier rises to disconnect the electrodes.
- The temperature of the control plate rises to 65 °C for 10 minutes to dry the gel.
- The control plate is cooled down ; when it reaches 50 °C, an audible beep signals that the migration module lid unlocks. The plate temperature remains at 50 °C until the lid is opened. Then, the temperature keeps decreasing until it reaches 20 °C (in less than 5 minutes) after which a new migration run may start.

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

## II. GEL PROCESSING SET-UP

1. Open the lid.
2. Remove the applicator(s) and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
4. Remove the dried gel film for further processing.
5. After each use, wipe the electrodes and the temperature control plate with a soft wet tissue.
6. 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. 5).
7. Place the gel holder into the Gel Processing / Staining Module.

**IMPORTANT:** Before starting the gel processing / staining program, check the following:

- 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.

8. Select "PROTEIN(E)/β1-β2" staining program from the instrument menu and start the run by pressing the "START" key.

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).

## III. GEL PROCESSING COMPLETION

1. Remove the gel holder from the compartment, open it and remove the dried gel.

*NOTE : After gel staining / destaining and before densitometry / scanning, a gel may be put through an additional wash step, if needed, to further clarify the gel background and to remove any residual stain that may appear as blue spots. Wash the gel using the "WASH ISOENZ/GEL" program.*

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.

*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 advised to include an assayed control serum (Control serum, SEBIA PN 4785) into each run of samples.

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

## RESULTS

### Values

Densitometer scanning of stained electrophoregrams yields relative concentrations (percentages) of individual protein zones.

Reference values (mean  $\pm$  2 SD) for individual major electrophoretic serum protein zones on HYDRAGEL PROTEIN(E) 15/30 gels have been established from a healthy population of 158 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.

FRACTION	Without HYDRAGEL CAPILLARYS/NEPHELOMETRIC Equivalency Values	With HYDRAGEL CAPILLARYS/NEPHELOMETRIC Equivalency Values
	HYRYS - GELSCAN - DVSE - PHORESIS	HYRYS - GELSCAN - DVSE - PHORESIS
Albumin	59.8 - 72.4 %	53.8 - 65.2 %
Alpha-1 globulins	1.0 - 3.2 %	1.1 - 3.7 %
Alpha-2 globulins	7.4 - 12.6 %	8.5 - 14.5 %
Beta globulins	7.5 - 12.9 %	8.6 - 14.8 %
Gamma globulins	8.0 - 15.8 %	9.2 - 18.2 %

It is recommended each laboratory establishes its own reference values.

### Interpretation<sup>1-15</sup>

SEBIA recommends interpreting the gel as soon as possible after the gel processing is completed. The quality of the gel deteriorates over time, depending on the storage conditions (including light, heat...). Each laboratory must define the optimum conditions between gel completion and gel interpretation based on the lab's environmental conditions.

The prolonged storage of the gels (in a dry place and away from light) should be done for archive purposes only.

Some serum samples may show a slight split which depends on the concentration and mobility of the alpha-2 zone protein components, see MIGRATION PATTERN.

- Some sera have different phenotypes (Haptoglobin, GC Globulin).
- Alpha-1 lipoprotein depends on the concentration and storage of the sample.

As an aid in interpretation of serum and urine protein electrophoregrams, see BIBLIOGRAPHY.

### Interference and Limitations

LDL and HDL lipoproteins are complexed components with a very variable natural electrophoretic mobility moving from the beta zone to the alpha-2 zone. In order to avoid difficulties of integration and interpretation, the HYDRAGEL gels are made of specific chemical composition which generally positions HDL in alpha-2 zone and LDL in beta zone.

The migration is very sensitive to the following parameters:

- sample storage,
- lipoproteins concentration,
- drug treatment (heparin for instance),
- dehydration level of the gel (storage of the gel),
- raw materials variations, even slight ones.

For these reasons, a slight anodic shift of these lipoproteins may be observed, which become more noticeable with a broadening or a slight splitting of the alpha-2 and/or beta zones.

- 1) The percentages of alpha-2 and beta fractions remain totally unchanged in spite of the slight splitting due to a variation of the electrophoretic mobility.
- 2) The characteristic shape of the beta-lipoprotein fraction (with an important focalisation and an irregular shape) may not lead to a misinterpretation, only the aspect has been changed.

### See SAMPLES ANALYSIS.

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 instruction for the preparation and storage of materials, and for the procedure were carefully followed.

Kit reagent Safety Data Sheets and information on cleaning and waste disposal, labeling and safety rules applied by SEBIA, packaging for the transportation of biological samples, and instruments cleaning are available on the SEBIA's extranet website : [www.sebia.com](http://www.sebia.com).

## PERFORMANCE DATA

### serum analysis

#### Reproducibility within run

Three (3) different samples were each run in 15 tracks on HYDRAGEL PROTEIN(E) 15/30 gels from the same lot. The means, SD and CV (n = 15) were calculated for each serum sample and each zone. The following table shows results for the 3 serum samples.

FRACTION	MEAN (%)			SD			CV (%)		
	1	2	3	1	2	3	1	2	3
Serum									
Albumin	70.4	68.8	65.9	0.2	0.7	0.6	0.3	1.0	0.9
Alpha-1	1.9	1.9	1.3	0.1	0.1	0.1	3.5	3.9	7.3
Alpha-2	9.9	9.4	7.6	0.2	0.2	0.2	1.6	2.6	2.4
Beta	10.6	8.6	7.8	0.4	0.3	0.1	3.3	3.5	1.8
Gamma	7.2	11.4	17.4	0.2	0.3	0.3	3.4	2.8	2.0

### Reproducibility between runs

Fifteen (15) different serum samples were each run during 5 different days using the same batch of HYDRAGEL PROTEIN(E) 15/30 gels. The means, SD and CV (n = 5) were calculated for each serum sample and each zone. The results were essentially the same for all samples. The following table shows the ranges of SD and CV representing all samples and a mean CV from the pooled CV's for all samples (n = 15).

FRACTION	SD	CV (%)	MEAN CV (%)
Albumin	0.2 – 0.9	0.3 – 1.4	0.8
Alpha-1	0.1 – 0.2	2.1 – 9.8	4.6
Alpha-2	0.1 – 0.3	0.9 – 3.1	1.8
Beta	0.1 – 0.3	1.1 – 3.8	2.0
Gamma	0.1 – 0.5	1.3 – 5.6	2.9

### Accuracy

Ninety (90) different samples (pathological and normal sera) were run on HYDRAGEL PROTEIN(E) 15/30 gels and another commercially available agarose gel system. The correlation parameters calculated for individual zones on HYDRAGEL PROTEIN(E) 15/30 gels vs. the comparative gel system (y-HYDRAGEL) were :

Fraction	Correlation Coefficient	y-Intercept	Slope	Range of % Values of samples used *
Albumin	0.983	3.673	0.942	54.9 - 72.9
Alpha-1	0.984	0.937	0.972	1.2 - 6.7
Alpha-2	0.984	0.635	0.977	7.6 - 16.5
Beta	0.953	-0.071	0.984	6.9 - 15.0
Gamma	0.976	-0.435	1.033	6.0 - 18.9

\* The percent values are as determined in the HYDRAGEL 15 & 30 PROTEIN(E) system.

Additionally, the analysis of 120 different normal and pathological samples, by electrophoresis on HYDRAGEL PROTEIN(E) 15/30 gel and on another commercially available system for proteins analysis by capillary electrophoresis, demonstrates a good correlation between both procedures, with a sensibility of 93.5 % and a specificity of 94.6 % for gammaglobulins, calculated according to the recommended method (Wendling, 1986).

### Sensitivity

A pathological serum sample with a monoclonal protein at 2.12 g/dL was serially diluted and the dilutions electrophoresed on HYDRAGEL PROTEIN(E) 15/30 gel. After visual inspection of the gel, the highest dilution with a discernible monoclonal band was 1/128 for the HYDRAGEL PROTEIN(E) 15/30 system. Thus the lowest concentration of a monoclonal protein was about 0.017 g/dL.

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

### Linearity

An albumin solution with 5.2 g/dL and a gammaglobulin solution with 3.1 g/dL (protein concentrations determined using spectrophotometry at 280 nm) were mixed within different proportions from 10 to 10 (100 % albumin solution + 0 % gammaglobulin solution, 90 % + 10 %, etc..., 0 % albumin solution + 100 % gammaglobulin solution) and the mixtures were electrophoresed with HYDRAGEL 30 PROTEIN(E) procedure.

The results demonstrated that the obtained percentage of each fraction is perfectly correlated with the theoretical percentage of each fraction within the mixture and that any variation may be detected with linearity using the HYDRAGEL 30 PROTEIN(E) procedure.

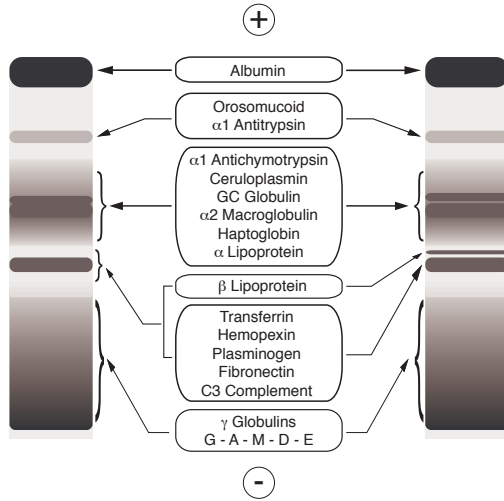
The HYDRAGEL 30 PROTEIN(E) procedure was determined to be linear for albumin and gammaglobulins fractions within the entire concentration range studied (between 0.0 and 5.2 g/dL of albumin and 3.1 g/dL of gammaglobulins).

### Concentrated urines analysis

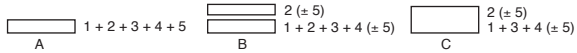
Results obtained with HYDRAGEL PROTEIN(E) 15/30 gels indicate a very good reproducibility within and between runs for concentrated urines samples after quantitative and qualitative analysis. Twenty eight (28) different samples (pathological and normal urines) were run on HYDRAGEL PROTEIN(E) 15/30 gels and another commercially available agarose gel system. There were no visually detectable differences among the two systems. The sensitivity of detection was determined from the highest serial dilutions of a monoclonal urinary protein giving a discernible band at 0.048 g/dL.



**MIGRATION PATTERN**



**Alpha-2 zone :**



- 1 =  $\alpha$ 2 Macroglobulin
- 2 = Haptoglobin
- 3 = Ceruloplasmin
- 4 = GC Globulin
- 5 =  $\alpha$ 1 Lipoprotein

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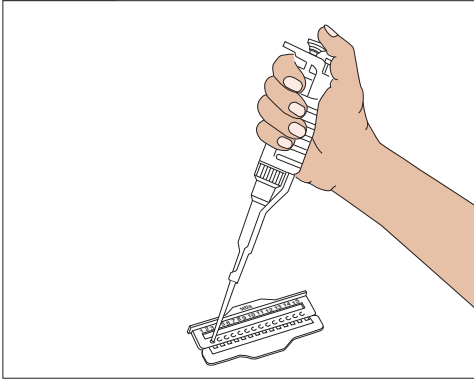
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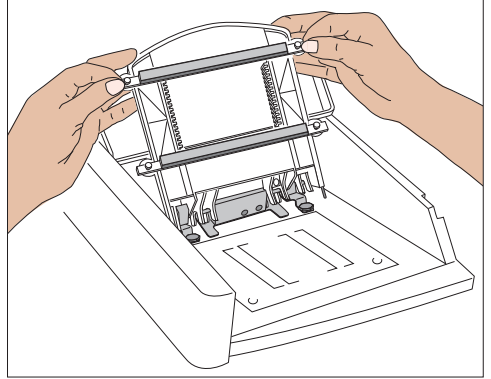
## SCHÉMAS / FIGURES

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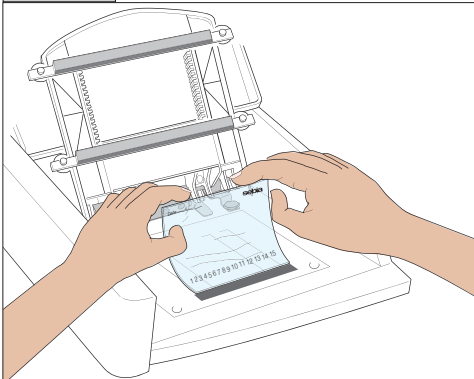
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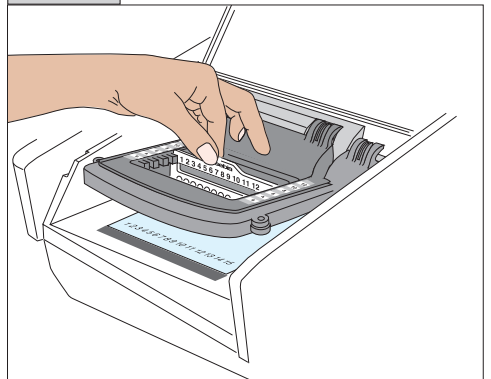
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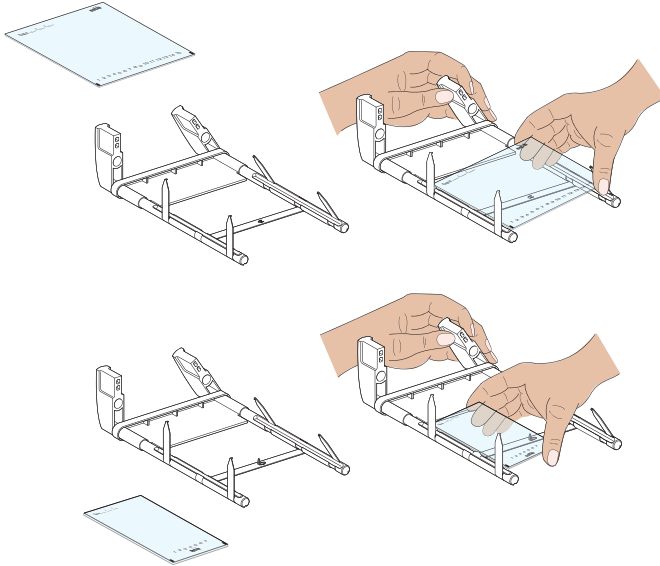


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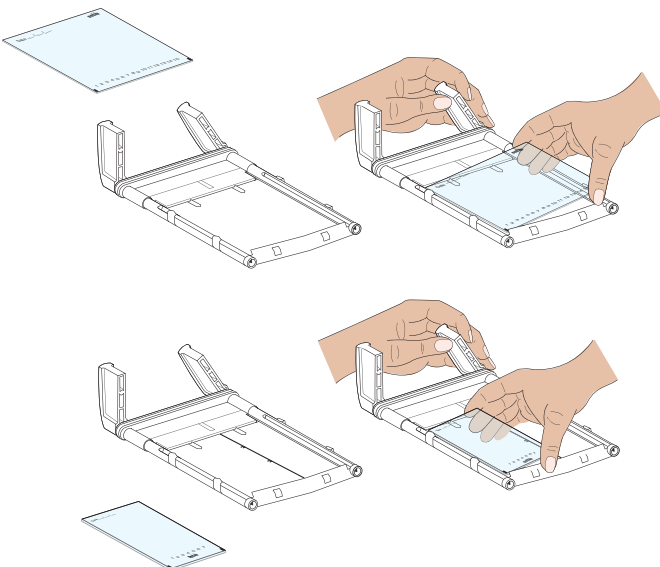
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5

## HYDRASYS



## HYDRASYS 2





**sebia**

Parc Technologique Léonard de Vinci  
CP 8010 Lisses - 91008 EVRY Cedex - France  
Tel.: +33 (0)1 69 89 80 80  
E-mail: [sebia@sebia.com](mailto:sebia@sebia.com)

[Contact your local Sebia offices](#)

