HYDRATEST 15 PROTEIN(E)
Ref. 4170

HYDRATEST 30 PROTEIN(E)
Ref. 4171
INTENDED USE

The HYDRAGEL PROTEIN(E) 15/30 gel is designed for separation of human serum proteins in human serum and urine by electrophoresis on alkaline buffered (pH 9.2) 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:
• 15 samples in the HYDRATEST 15 PROTEIN(E) kit,
• 30 samples in the HYDRATEST 30 PROTEIN(E) kit.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST1-15

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 pattern 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 HYDRATEST 15 PROTEIN(E) AND HYDRATEST 30 PROTEIN(E) KITS

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4170</th>
<th>PN 4171</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
<td>10 gels</td>
</tr>
<tr>
<td>Buffered Strips (ready to use)</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
</tr>
<tr>
<td>Staining solution diluent (stock solution)</td>
<td>1 vial, 60 mL</td>
<td>1 vial, 60 mL</td>
</tr>
<tr>
<td>Amidoblack Stain (stock solution)</td>
<td>1 vial, 20 mL</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10</td>
<td>2 packs of 10</td>
</tr>
<tr>
<td>Filter Papers</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
</tr>
</tbody>
</table>

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 9.2 ± 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.

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: tris-barbital buffer pH 9.2 ± 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. STAINING SOLUTION DILUENT

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

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 visqueous 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: acid solution pH ≈ 2; amidoblack, 0.4 g/dL; ethylene-glycol, 6.7 %; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Harmful if swallowed.

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. APPLICATORS

Use
Precut, single use applicators for sample application.

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

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 or refrigerated.

REAGENTS REQUIRED

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: citric acid, 0.05 g/dL.

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 vials.
Working destaining 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 destaining solution close to a heat source.

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.
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: 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
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 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.

3. FLUIDIL

Preparation
Fluidil (SEBIA, PN 4587, 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.

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.
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 102 mm gels at 570 nm or with a yellow filter, e.g., HYRYS SEBIA, DVSE 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 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.
   Upon storage at 2 to 8 °C or after freezing, some sera (particularly those containing 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 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 (e.g., 10 minutes at 3,000 rpm) or filtration (e.g., 0.45 µm syringe filter).

Sample to avoid
- Do not use 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.
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 INSTRUCTION MANUAL.

I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator for 15 samples, or two applicators 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 «15/30 PROTEIN(E)» 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 the applicator(s) from the wet chamber. Handle it (them) by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - For 15 samples analysis, place the applicator into position No 6 on the carrier.
   - For 30 samples analysis, place the two applicators each into position Nos 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 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.

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 «PROT./B1-B2/Hb» 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).

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 at 570 nm or with a yellow filter.

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

## RESULTS

### Quality control

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

### Values

Densitometer scanning (at 570 nm) of stained electrophoregrams yields relative concentrations (percentages) of individual protein zones.

Normal values (mean ± 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.

### Interpretation

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

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 informations on waste products elimination are available from the Technical Service of the supplier.

## 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.

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

#### 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.

**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 15/30 PROTEIN(E) system. Thus the lowest concentration of a monoclonal protein was about 0.017 g/dL.

**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 used.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation Coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.87</td>
<td>0.06</td>
<td>0.412</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.87</td>
<td>0.06</td>
<td>0.412</td>
</tr>
<tr>
<td>Alpha-2</td>
<td>0.87</td>
<td>0.06</td>
<td>0.412</td>
</tr>
<tr>
<td>Beta</td>
<td>0.87</td>
<td>0.06</td>
<td>0.412</td>
</tr>
<tr>
<td>Gamma</td>
<td>0.87</td>
<td>0.06</td>
<td>0.412</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**

(2) 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.
MIGRATION PATTERN

Alpha-2 zone:

1 = α2 Macroglobulin
2 = Haptoglobin
3 = Ceruloplasmin
4 = GC Globulin
5 = α1 Lipoprotein

A = 1 + 2 + 3 + 4 + 5
B = 2 (± 5)
C = 2 (± 5)

2 (± 5)
1 + 2 + 3 + 4 (± 5)
1 + 3 + 4 (± 5)