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HYDRAGEL ISO-PAL K20

Ref. 3022

CE

2004/05

INTENDED USE

The HYDRAGEL ISO-PAL K20 kit is designed for the identification and quantification of alkaline phosphatase (AP) isoenzymes in human serum. The serum samples are electrophoresed on alkaline buffered (pH 9.1) agarose gels. The separated AP isoenzymes are visualized using a specific chromogenic substrate. The dried gels are ready for visual examination and densitometry to obtain accurate relative quantification of individual zones.

Each agarose gel is intended to run 3 samples in the HYDRAGEL ISO-PAL K20 kit.

For *In Vitro* Diagnostic Use.

PRINCIPLE OF THE TEST

The alkaline phosphatase is a metalloglycoprotein with a monophophoesterase activity. It catalyzes the hydrolysis of monophosphoric esters, which is activated by magnesium ions in alkaline medium.
This enzyme is found in many human tissues, e.g., liver, bone, kidney, intestine and placenta. Its elevation is evident in a variety of hepatic, non-hepatic conditions as well as in normal adolescent growth. The enzymes of alkaline phosphatase are coded by 3 structural genes: 2 genes code respectively for the placental and intestinal isoenzymes. The 3rd gene called "non specific tissue gene" is expressed in a variety of tissues such as bone, the liver and kidney.
The individual AP isoenzymes can normally be separated by electrophoresis according to the charge differences. However, the electrophoretic mobilities of the liver and bone isoenzymes are induced only by glycosylation or post-translational modifications and therefore are quite similar. They must be separated with a special treatment of the sample. Quantitation of the AP isoenzymes helps to identify the tissues responsible for the elevation.
Electrophoresis of human serum on HYDRAGEL ISO-PAL gels resolves nearly all alkaline phosphatase isoenzymes except the liver 1 (L1) and bone (B), and the placental 1 (P1) when present, migrate together in a single broad fraction.
From anode to cathode, the migration pattern is as follows (see the figure "Migration Patterns"): L1 + B (+ P1) ; liver 2 (L2, this isoenzymes is also referred to as macrohepatic or fast liver) ; intestinal 1, 2 and 3 (I1, I2 and I3). The placental 2 (P2), when present, is located between I1 and I2. Additional fraction can be seen in some highly icteric samples close to the application point. It is a complex of alkaline phosphatase and lipoproteins called lipo-ISO-PAL (or ultra fast, UF, in other procedures).
To overcome the inherent difficulties in separating L1 and B isoenzymes, various sample treatments have been explored and some of them routinely used, e.g., differential inactivation by heat, urea or amino acids, precipitation with specific antiserum, differential sensitivity to enzymatic desialation with neuraminidase, etc.
All AP isoenzymes are sialated to certain degree except the intestinal isoenzymes are devoid of sialic acid. The SEBIA system utilizes the different degree of sialation of L1 and B isoenzymes to separate them. Wheat germ lectin (wheat germ agglutinin, WGA) presents a strong affinity for the sialic acid residues and consequently binds preferentially to the bone isoenzyme that is sialated to the highest degree of all. To perform the test, each sample is applied in duplicate. In the course of migration, one of the sample duplicates passes through lectin deposited anodally from the sample's point of application (the lectin itself shifts only slightly cathodally). The interaction between WGA and the passing AP isoenzymes is a reversible reaction:

Sialated isoenzyme + WGA $\underset{2}{\overset{1}{\rightleftharpoons}}$ sialated isoenzyme/WGA complex

Under the conditions chosen for the electrophoresis, the interaction of WGA with L1, L2, P1 and P2 isoenzymes is weak and the above reaction shifts in the direction 2. Hence, these isoenzymes are only slightly slowed down compared to their position on the pattern run without any lectin. On the other hand, the interaction with the B isoenzyme rich in sialic acids is strong and sufficient to precipitate the majority of B as a sharp band close to the lectin application point. When the binding capacity of the lectin is exceeded (e.g., the bone isoenzyme is highly elevated as in bone cancers and active bone metabolism in growing age), the precipitation might be incomplete and occasionally an anodic smear can be observed. The smear does not affect the densitometry on the "lectin" profile (see "Interpretation"). The separated AP isoenzymes are visualized using a specific chromogenic substrate, 5-Bromo-4-Chloro-3-Indolyl Phosphate / Nitro Blue Tetrazolium in Aminomethyl Propanol (AMP) buffer, pH 10.0.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL ISO-PAL K20 KIT

ITEMS	PN 3022*
Agarose Gels (ready to use)	10 gels
HYDRAGEL ISO-PAL Buffer (stock solution)	3 vials, 100 mL each
Lectin (freeze-dried)	1 vial
Substrate (ready to use)	1 vial, 20 mL
Chromogen (stock solution)	1 vial, 10 mL
Destaining solution (stock solution)	1 vial, 100 mL
Applicators (ready to use)	2 packs of 10 (7 teeth)
Filter Papers - Thin	1 pack of 10
Filter Papers - Thick	1 pack of 10

** The HYDRAGEL ISO-PAL K20 kit contains applicators with 7 teeth. Since each sample is applied in duplicate, 3 unknown samples can be assayed per gel. The remaining track can be used for control.*

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, 1 g/dL ; alkaline buffer pH 9,1 ± 0.05 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The gels contain 0.10 % sodium azide. Do not ingest ! If ingested, consult physician immediately !

Use

Support medium for alkaline phosphatase isoenzymes electrophoresis.

Storage, stability and signs of deterioration

Store the gels horizontally in the original protective packaging preferentially refrigerated (2 to 8 °C). (The arrow on the front of the kit box must be pointing upwards).
Avoid obvious temperature fluctuations during storage (e.g., do not store close to a window or a heat source). The gels are stable until the expiration date indicated on the kit package or the gel package labels. DO NOT FREEZE.
Discard gel 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. HYDRAGEL ISO-PAL BUFFER

Preparation

Each vial of the stock buffer solution to be diluted up to 1 liter with distilled or deionized water.
After dilution, the working solution contains: alkaline buffer pH 9.3 ± 0.2 ; sodium azide ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Stock buffer contains 0.50 % 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

Electrophoresis buffer.

Storage, stability and signs of deterioration

Store stock buffer solution at room temperature or refrigerated. It is stable at least to the expiration date indicated on the kit package or buffer vial labels.
Diluted buffer solution is stable for one year at room temperature in a closed bottle.
Discard diluted buffer if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

3. LECTIN*

Preparation

The lectin vial contains Wheat Germ Agglutinin (WGA) in a stabilized lyophilized form.
Reconstitute the lectin vial with exactly 0.5 mL of saline. Close the vial. Mix gently and let it stand at room temperature for 5 minutes. Then, mix gently to obtain clear solution of lectin that is ready to use.

Use

For the treatment of the migrating serum samples during electrophoretic separations.

Storage, stability and signs of deterioration

Store the lyophilized lectin refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or lectin vial label.
After reconstitution, the lectin solution must be stored at – 20 °C ; for that purpose, we recommend to prepare 50 µL aliquots. Alternatively, the entire volume may be stored at – 20 °C ; it can undergo ten (10) freezing/defreezing cycles without any adverse effects on performance as long as the solution is not exposed to room temperature for more than 15 minutes during each cycle.

4. SUBSTRATE

Preparation

Substrate is ready to use. It contains: BCIP (5-Bromo-4-Chloro-3-Indolyl Phosphate) in Aminomethyl Propanol buffer (AMP) pH 10.0, additives, nonhazardous at concentrations used, necessary for optimum performance.
Prepare the visualization solution away from light just before use : add 0.5 mL of chromogen to 2 mL of substrate and mix.

Use

For the preparation of the visualization solution.

Storage, stability and signs of deterioration

Store substrate at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or substrate vial label.
The substrate may show mild crystallisation without any adverse effects on its performance.

5. CHROMOGEN

Preparation

Chromogen is ready to use. It contains: NBT (Nitroblue Tetrazolium) in water.

Use

For the preparation of the developing solution as described in paragraph 4.

Storage, stability and signs of deterioration

Store chromogen at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or chromogen vial label.

6. DESTAINING SOLUTION

Preparation

Vial of destaining solution to be diluted up to 100 liters with distilled or deionized water. It is convenient to prepare a 1/1000 dilution of only a small aliquot of the stock solution, e.g., dilute 1 mL stock solution up to 1 liter.
After dilution, the working destaining solution contains: citric acid, 0.05 g/dL.

Use

For washing the gels after incubation with ISO-PAL substrate to remove its excess.

Storage, stability and signs of deterioration

Store 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.
Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
Do not add any sodium azide. Do not wash more than two gels in each 300 mL of destaining solution.

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.

7. APPLICATORS

Use
Precut, single use applicators for sample and lectin solution application onto gel.

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

8. THIN 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.

9. THICK FILTER PAPERS

Use
Precut, single use, thick absorbent paper pads for blotting the gel after enzymatic visualization.

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

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

REAGENT REQUIRED BUT NOT SUPPLIED

SALINE

Preparation
Make 0.15 M (0.9 g/dL) NaCl solution in distilled or deionized water.

Use
To reconstitute the lectin vial.

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

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. Power supply: GD 61 D SEBIA, PN 1300 ; GD 251 D SEBIA, PN 1301 ; MG 300 SEBIA, PN 1302 or MG 500 SEBIA, PN 1303.
2. HYDRAGEL K20 APPLICATOR SEBIA, PN 1409, containing the HYDRAGEL K20 applicator carrier.
3. HYDRAGEL ENZ K20 accessory kit, SEBIA PN 1422, containing the template carrier K20 and the reagent application template ENZ 4 mL.
4. Wet Storage Chamber, PN 1270.
5. Electrophoresis chamber: K20 SEBIA, PN 1400.
6. Tanks, Incubation Boxes and Gel Holders for processing of gel plates: HYDRAGEL K20 Accessory Kit SEBIA, PN 1420.
7. Pipettes: 10 µL, 200 µL, 1 mL and 5 mL.
8. Incubator-Dryer: IS 80 SEBIA, PN 1430.
9. Densitometer / scanner capable of scanning 82 x 51 mm gels at 570 nm (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.
10. Quality control materials.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh serum samples are recommended for analysis. Samples must be collected according to established procedures used in clinical laboratory testing. Store samples at 2 to 8 °C as soon as possible after collection, and for up to one week.

Sample preparation
Use neat serum. Dilute serum samples with saline to achieve a total alkaline phosphatase activity of about 600 IU/L when this activity is > 600 IU/L. See Note under “PERFORMANCE DATA/Linearity”.

Samples to avoid
Do not use hemolyzed samples. The erythrocyte enzymes may interfere in the reaction.
Do not use samples that contain an inhibitor of alkaline phosphatase enzymatic activity such as EDTA, citrate or oxalate.

PROCEDURE

I. MIGRATION SET UP

1. Place the HYDRAGEL K20 applicator carrier on a flat surface (Fig. 1) and raise the part of the applicator carrier with the numbered notches.
2. Pool 120 µL distilled or deionized water on the lower third of the frame printed on the HYDRAGEL K20 applicator carrier.
3. Unpack the HYDRAGEL agarose gel plate.
4. 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.
5. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 2).
6. Bend the gel and lower it down onto the water pool (Fig. 2). 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 the applicator carrier with the numbered notches down to the intermediate position with the switch in high position.
8. It is necessary to use two applicators per each HYDRAGEL 7 ISO-PAL gel to apply serum samples and lectin solution.

   Place applicators, two of each HYDRAGEL 7 ISO-PAL (7 teeth for 3 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 3).
   - First applicator: apply 10 µL of each serum sample in 2 adjacent wells.
   - Second applicator: apply 10 µL lectin solution in each even-numbered well.
   - Load each applicator within 2 minutes. Assayed control serum may be applied into the last well of each applicator.
   - Use the applicators without any delay. For later use (up to 8 hours), place the applicators into the wet storage chamber with the teeth up [handle them by the plastic tooth protection frame], keep the entire chamber under refrigeration and set-up the gel plate onto the HYDRAGEL K20 applicator carrier just before use.

     See wet chamber package insert for further details.
9. Snap off the applicators teeth's protection frame.
10. Place the applicator with serum samples into position No. 3 on the applicator carrier.
11. Place the applicator with lectin into position No. 4 on the applicator-carrier.
12. Block the applicators on the left side of the carrier, for example.

    IMPORTANT: The numbers printed on the sample applicators must face the operator (Fig.4).
13. Lower the applicator-carrier so that applicators contact the gel surface. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
14. After 15 minutes, turn the switch to rise up the applicators, remove the applicators and discard.
15. Put the gel into an appropriate electrophoresis chamber, according to the polarity indicated on the gel. When using SEBIA K20 chamber, place the HYDRAGEL on the bridge with the gel side facing down ; the gel should dip about 1 cm into the buffer on each side.

    See K20 chamber package insert for further details.
16. Plug the chamber to the power supply.

MIGRATION CONDITIONS	SEBIA K20
Volume of buffer per compartment	150 mL
Total buffer volume	300 mL
Migration time	45 minutes
Constant voltage	120 V
Initial current (per gel)	10 ± 2 mA

17. After migration, unplug the chamber and remove the gel plate.

II. PREPARATION FOR INCUBATION WITH ISO-PAL SUBSTRATE

1. Place the template carrier on a flat surface (Fig. 5) and remove the cover.
2. Pool 120 µL distilled or deionized water, on the lower third of the frame printed on the template carrier.
3. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 6).
4. Bend the gel and ease it down onto the water pool (Fig. 6). 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.
5. Set up the reagent application template ENZ 4 mL on the template carrier as follows (Fig. 7) :
   - Position the application template on the anchoring clips.
   - Hold the handle of the template and lower the template onto the gel.
6. After having mixed chromogen and substrate (Ref. paragraph 4) in the vial, apply immediately 2.5 mL of ISO-PAL substrate solution, as follows (Fig. 8):
   - Hold the pipette vertically.
   - Lightly press the tip of the pipette into the hole of the template.
   - Carefully and progressively inject the reagent without introducing air bubbles under the template.
7. Close the cover of the template carrier (Fig. 9).
8. Place the template carrier in the incubator-dryer at 37 °C for 30 minutes, and at 51 °C for 30 minutes.

III. SUBSTRATE ELIMINATION AND FILTER PAPER APPLICATION

1. After incubation, remove the template carrier from the incubator-dryer and place it on a flat surface.
2. Open the cover.
3. Remove the remaining substrate solution :
   - Hold the pipette vertically and lightly press the tip of the pipette into the well.
   - Carefully and progressively withdraw the reagent.
4. Remove the template:
   - Grasp the handle of the template.
   - Raise the template and remove it.
5. Leave the gel on the plate of the template carrier.

6. Apply one thick filter paper on the gel for 3 minutes:
   - Slope the filter paper at about 45 °.
   - Align the lower side of the filter paper with the edge of the gel.
   - Lower the filter paper onto the gel.
   - Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
7. Remove the filter paper. Dry the gel at 51 °C for 3 minutes.
8. Rinse the template with distilled water or alcohol and dry it thoroughly with soft absorbent paper. Prior to re-use, ensure the template is completely dry.

IV. WASHING AND DRYING STEPS

1. Place the gel into a gel holder (supplied with SEBIA HYDRAGEL K20 Accessory Kit).
2. Immerse the gel vertically in the destaining solution for 10 minutes. Keep the gel away from light.
3. Dry the gel completely at 51 °C.

V. EVALUATION

Evaluate the gels visually. When required, scan using a densitometer / scanner with a yellow filter or at 570 nm.

RESULTS

Quality Control

It is advised to include a commercially available control serum and/or a known assayed serum into each run of samples.

Values

The enzymatic activity of each fraction can be calculated from the densitometric percent values for each fraction and the total AP activity. In order to quantify all AP isoenzymes, scan both the "with" and "without" lectin tracks.
The values of isoenzymes L1 and P1 are determined on the lectin track. The value of B isoenzyme is obtained by subtracting the densitometric values of L1 and P1 fractions obtained from the lectin track, from the value of the L1 + B (+ P1) fraction of the "without" lectin track. All other fractions, L2, I1, I2, I3, P2 and the lipo-ISO-PAL complex are determined on the track without lectin.

Normal values

The normal values of the individual isoenzymes vary with age, sex, hormonal state (pregnancy, menopause, puberty) and medications. In adult subjects, from 20 to 50 years old, the values are nearly independent of age. In older population, the total AP tends to increase with age due to physiological factors such as menopause and the process of aging. In children, total AP is increased due to predominant bone isoenzyme. Normal values for the major electrophoretic alkaline phosphatase isoenzymes on HYDRAGEL ISO-PAL gels have been established from a healthy population of 100 adults (50 men and 50 women, 20-50 years old) and 50 children (2-18 years old):

	WOMEN		MEN		CHILDREN	
	%	IU/L	%	IU/L	%	IU/L
total AP *		≤ 80		≤ 122		≤ 410
L1	18 - 72	≤ 45	15 - 71	≤ 64	1 - 31	≤ 51
B	20 - 74	≤ 44	23 - 75	≤ 73	62 - 100	≤ 370
L2	1 - 14	≤ 8	1 - 9	≤ 8	1 - 7	≤ 19
I1, I2, I3	Intestinal isoenzymes are absent in about 60 % of normal subjects ; when present they do not exceed 14 %					

** The above total AP activity has been determined at 37 °C in AMP buffer.*

It is recommended each laboratory establish its own normal values.

The above activity values (IU/L) represent maximum normal values. The normal total AP activity values (IU/L) depend on the procedure and temperature used to determine the activity (commercial kits and laboratory requirements may vary in this respect). The activities per fraction must be based on the total AP activity and the assay conditions specified.

Interpretation

Interpretation of the electrophoretic patterns requires the knowledge of the total sample AP activity, sex and age of the patient. All sialated fractions are slowed down to a various degree on the lectin track compared to the "without" lectin track. Only intestinal fractions are not affected by the lectin.
On the lectin track, the bone fraction usually forms a single sharp band close to the application point. Occasionally, in addition to the sharp band, an anodic smear can be observed. This phenomenon generally results from exceeding the binding capacity of the lectin and consequently, incomplete precipitation, e.g., when the bone isoenzyme is highly elevated as in bone cancers and active bone metabolism. In no case the smear extends to the migration position of L1 or P1 so that these two fractions can be accurately quantified by densitometry on the "lectin" profile.

See the migration pattern as an aid in identification of individual isoenzymes.

1. Normal serum

The liver (L1) and bone isoenzymes are the two fractions usually found in sera from healthy subjects. On HYDRAGEL 7 ISO-PAL, they migrate superimposed on the track without lectin and are completely separated on the lectin track.
One or several (up to three) intestinal fractions can also be seen in normal serum. At normal total AP activity level, the presence of the intestinal fraction(s) has not been associated with any pathological condition. Non-fasting subjects and B or O blood group subjects exhibit this intestinal isoenzyme more often.

2. Particular cases

Liver isoenzymes

There are two liver isoenzymes, L1 and L2. The L1 fraction is the most anodic of all isoenzymes and the more significant of the two liver isoenzymes. L1 is increased in some non-malignant diseases (such as cholestasis, cirrhosis, viral hepatitis and in various biliary and hepatic pathologies). It is also increased in malignancies with hepatic metastasis, in cancer of the lungs and digestive tract and in lymphoma.

The L2 fraction (also called macrohepatic or fast liver) also exists in healthy subjects but at low concentration (< 8 IU/L). It could be of membranous origin and associated with lipoproteins. An increase of L2 may occur in cholestasis and biliary diseases (e.g., cirrhosis, viral hepatitis) and in malignancies (e.g., breast, liver, lung, prostate, digestive tract) with liver metastasis. On HYDRAGEL 7 ISO-PAL gels, L2 clearly migrates between the (L1 + B) and intestinal fractions.

Bone isoenzyme

This is a significant isoenzyme present in all samples. The bone fraction has to be interpreted with respect to age since its elevation is physiologically normal during the growth age. Increase of bone isoenzyme is primarily associated with the following conditions:

- In malignancies such as breast cancer with bone or liver metastasis. Also in osteosarcoma and lymphoma with or without metastasis.
- In rheumatismal diseases, hyperparathyroidism, Paget disease and rachitism.
- In transient hyperphoshatasemia in infancy that is associated with a particular liver fraction (more anodic than L1). In such case the total AP is highly elevated (> 2000 IU/L).
- When associated with an increase of other fraction (e.g., L1, L2) it may indicate malignancy of rectum, lung and prostate (with bone and liver metastasis).

Intestinal isoenzymes

Mobility of adult intestinal isoenzymes is the same on both tracks. They can be seen as 1, 2, or 3 fractions. About 40 % of normal subjects possess intestinal isoenzyme. Increased activity can be found in some diseases such as liver cirrhosis, diabetes and chronic renal failure.

Placental isoenzyme

There are always two forms of placental isoenzymes: the major form P1, and the minor form P2, representing about 90 % and 10 %, respectively. It is found during pregnancy and in some malignant diseases, primarily in ovarian cancers, as well as sarcomas, pancreatic and stomach cancers. Increase can be also due to heavy smoking (release from the lungs).

Immunoglobulin complex or macro-alkaline phosphatase

This fraction is rarely found. It is an immunoglobulin / AP complex. It remains very close to the application point on both tracks.

Alkaline phosphatase / LP complex

This is a lipoprotein / L2 complex. It remains very close to the application point on both tracks. It may be found in biliary obstructive diseases.

Atypical isoenzymes

Isoenzymes with atypical migrations, such as Nagao, Regan and Kasahara, are difficult to identify.

Limitations

See also SAMPLES FOR ANALYSIS.

No interference with HYDRAGEL ISO-PAL procedures was detected due to the sample's high concentration of cholesterol (≤ 360 mg/dL), triglycerides (≤ 1560 mg/dL) and bilirubin (≤ 10 mg/dL).

NOTE: On electrophoresis, the yellow coloration of icteric samples due to billirubin is present in two fractions. One co-migrates with albumin (i.e., is by 10 mm faster than L1 isoenzyme) and the other forms a diffused fraction at level of L2. The yellow coloration is virtually eliminated during the blotting and washing steps of the visualization procedure. The residual bilirubin does not interfere with the scanning that is performed with yellow filter or at 570 nm.

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

SEBIA's HYRYS densitometer was used for all densitometric measurements.

Within gel reproducibility

Three different serum samples (A, B & C) were electrophoresed using HYDRAGEL ISO-PAL K20 procedure on gels from the same lot. The samples were : A - normal serum ; B – serum with intestinal fractions I1 and I2 ; C – serum with elevated liver fractions L1 and L2. Each sample was applied in all tracks of a single gel. The electrophoregrams were evaluated by densitometry.

The following table shows the means, SD, CV for each alkaline phosphatase fraction from each serum sample for each gel.

PARAMETER	% L1	% B	% L2	% I1	% I2	% I3
Sample A						
MEAN	59.7	35.0	5.2	/	/	/
SD	1.4	1.5	0.3	/	/	/
CV (%)	2.3	4.4	5.9	/	/	/
Sample B						
MEAN	40.3	31.1	5.3	6.4	14.0	2.9
SD	0.6	0.8	0.2	0.1	0.4	0.2
CV (%)	1.4	2.7	3.3	1.5	2.7	7.3
Sample C						
MEAN	58.5	8.9	32.6	/	/	/
SD	0.6	0.9	0.4	/	/	/
CV (%)	1.0	9.7	1.3	/	/	/

Between gels reproducibility

Seven different serum samples were run each using HYDRAGEL ISO-PAL K20 procedure on 10 gels from the same lot. The means CV, SD and CV (n = 10) were calculated for each serum sample and each alkaline phosphatase fraction. 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 calculated from the pooled CV's for all samples.

FRACTION	SD	CV (%)	MEAN CV (%)
L1	0.3 - 2.0	0.6 - 18.2	5.3
P1	0.9 - 1.7	2.6 - 3.3	2.9
B	0.5 - 1.8	0.6 - 8.5	6.2
L2	0.1 - 0.5	1.5 - 21.3	8.4
P2	0.3 - 0.8	3.0 - 7.9	5.4
I1	0.2	2.6 - 10.0	6.3
I2	0.2	1.7	1.7
I3	0.2	6.2 - 21.6	13.9

Accuracy - Comparative study

Sixty (60) different serum samples (normal and pathological) were analyzed using SEBIA's HYDRAGEL ISO-PAL K20 kit and another, commercially available test for electrophoretic determination of alkaline phosphatase isoenzymes. There was a perfect agreement between the two tests in visual detection of the alkaline phosphatase isoenzymes. The results of linear regression analysis of the densitometric values of L1, Bone and L2 fractions (n = 60) are tabulated below (y = SEBIA values).

PARAMETER	Correlation Coefficient	y-intercept	Slope	Range of IU/L values (SEBIA's test)
L1 fraction	0.991	-0.241	0.979	17 - 603
Bone fraction	0.974	-2.442	1.016	4 - 401
L2 fraction	0.992	3.551	1.003	0 - 277

Sensitivity

One serum sample with I1, I2 and I3 fractions and another one with P1 and P2 fractions were serially diluted and the dilutions electrophoresed using HYDRAGEL ISO-PAL K20 procedure. The lowest detected activity of the individual isoenzymes by densitometry was 3.0 IU/L.

Linearity

Serial dilutions of 2 samples containing the L1/L2 fractions and L1/bone fractions were tested, from, respectively, total alkaline phosphatase activity of 1000 to 10 IU/L and 600 to 50 IU/L using HYDRAGEL ISO-PAL K20 procedure. The system was linear in the entire range studied.

NOTE: The maximum value of the narrower linearity range studied (600 IU/L) was taken as approximate target value for diluting samples with high total AP activity.

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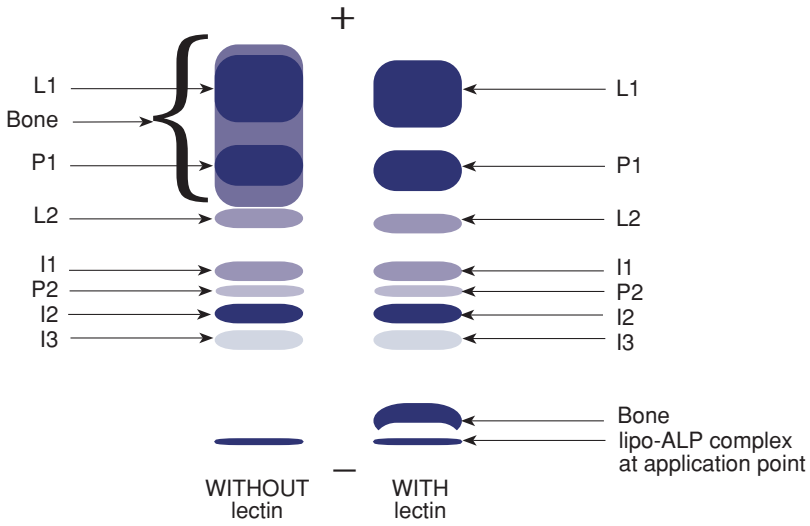
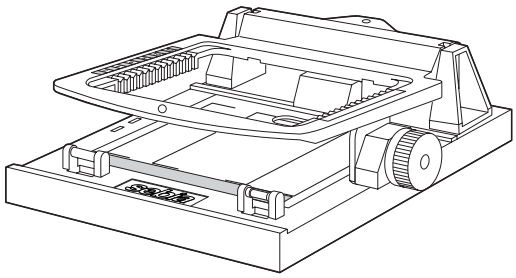
MIGRATION PATTERNS



SCHÉMAS / FIGURES

Figure 1



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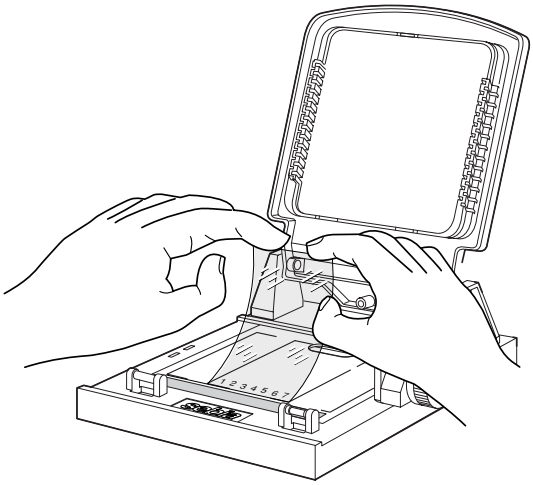

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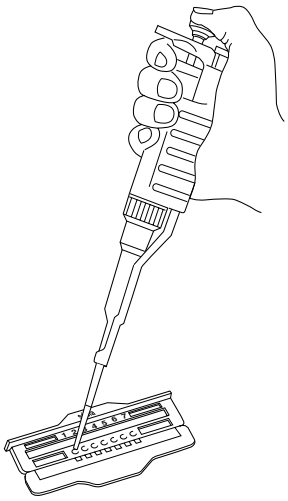

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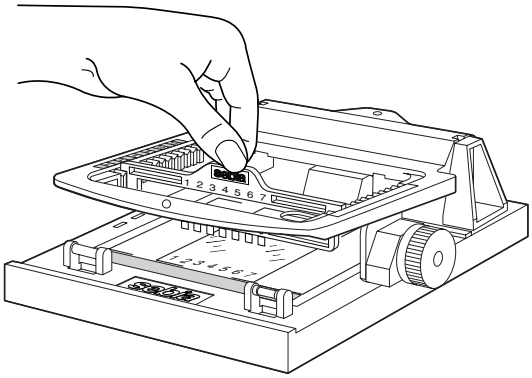

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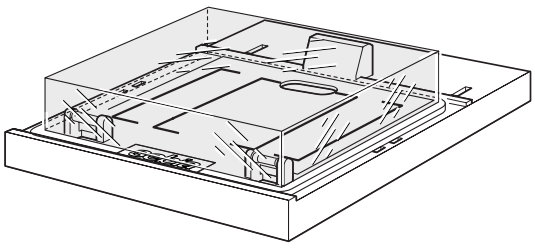

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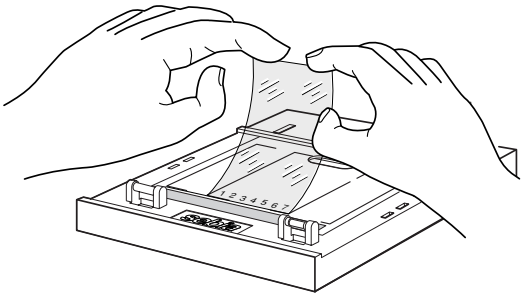

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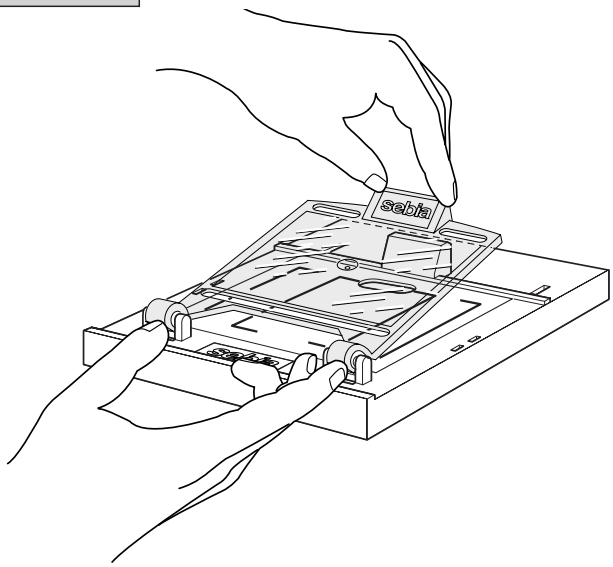

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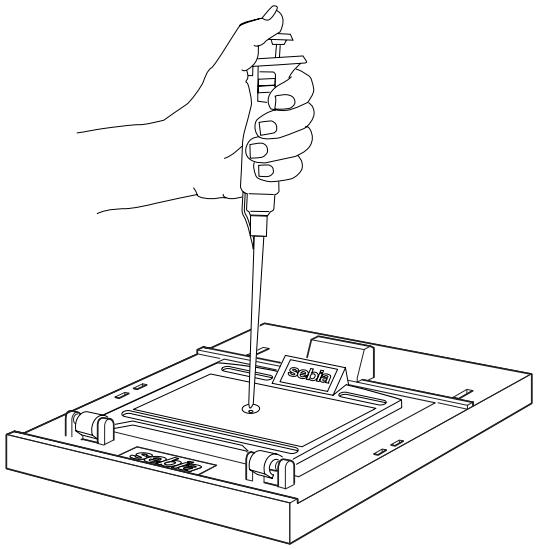

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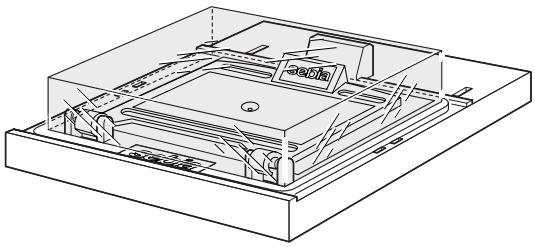

sebia

Parc Technologique Léonard de Vinci
Rue Léonard de Vinci
CP 8010 Lisses
91008 EVRY CEDEX - FRANCE

sebia Benelux s.a. / n.v.
Rue de la Fusée, 62
Raketstraat, 62
1130 Bruxelles / Brussel
BELGIQUE / BELGIË

sebia GmbH
Michael Henkel Straße 4-6
36043 Fulda
DEUTSCHLAND

sebia Hispania S.A.
C/Sicilia, 394
08025 Barcelona
ESPAÑA

sebia Italia s.r.l.
Via Antonio Meucci, 15/a
Località Ponte a Ema
50012 Bagno a Ripoli, Firenze
ITALIA

sebia Inc.
400-1705 Corporate Drive
Norcross, Georgia 30093
U.S.A.