HYDRAGEL 7 ISO-CK

Ref. 4111

HYDRAGEL 15 ISO-CK

Ref. 4131

HYDRAGEL 30 ISO-CK

Ref. 4137

IVD

CE

2004/11

INTENDED USE

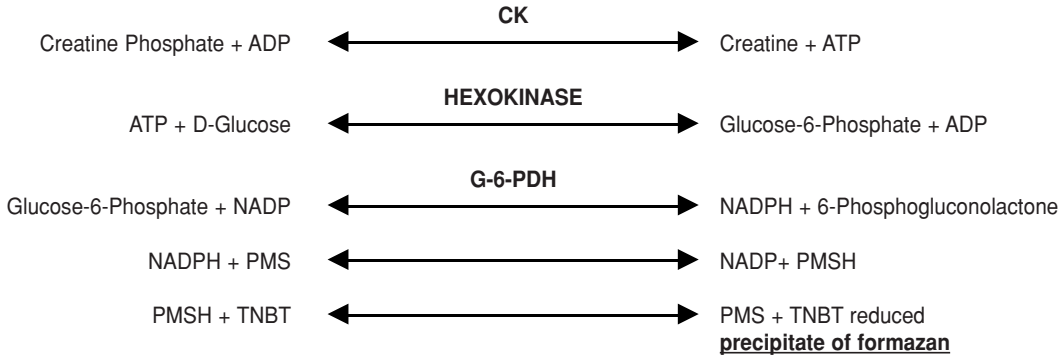
The HYDRAGEL 7, 15 and 30 ISO-CK kits are designed for the identification and quantification of the three creatine kinase (CK) isoenzymes in human serum by electrophoresis on alkaline buffered (pH 8.4) agarose gels. The kits are used in conjunction with the semi-automated HYDRASYS electrophoresis system. The resulting gels are ready for visual examination and densitometry to obtain accurate relative quantification of individual zones.

Each agarose gel is intended to run:
- 7 samples in the HYDRAGEL 7 ISO-CK kit,
- 15 samples in the HYDRAGEL 15 ISO-CK kit,
- 30 samples in the HYDRAGEL 30 ISO-CK kit.

For *In Vitro* Diagnostic Use.

PRINCIPLE OF THE TEST

Creatine kinase isoenzymes consist of two subunits: M («muscle») and B («brain»), assembled in dimers. The three resulting combinations constitute the three isoenzymes: MM, principally located in cardiac and skeletal muscles, MB in cardiac muscle and BB in cerebral tissues.
Each subunit has a specific electric charge which confers characteristic mobility to the individual CK isoenzymes. On HYDRAGEL 7 ISO CK/LD and HYDRAGEL ISO CK/LD 15/30 gels, the BB fraction is the most anodic, the MM fraction is the most cathodic and the MB is intermediary.
The major value of CK isoenzyme electrophoresis is the confirmation of myocardial infarction (MI). The MB concentration increases 4 to 8 hours after myocardial infarction. The CK isoenzyme analysis is generally run in tandem with LD (lactate dehydrogenase) isoenzymes and/or other early cardiac markers to confirm or rule out the diagnosis of MI, assess its severity and monitor the patient's condition.
All CK isoenzymes catalyze the same reaction that is utilized in their visualization. In the HYDRAGEL 7, 15 and 30 ISO-CK kits, the serum samples are electrophoresed and the separated CK isoenzymes are visualized using a specific chromogenic substrate according to the following reactions :



The amount of resulting formazan precipitate is proportional to the CK enzymatic activity.

ABBREVIATIONS :
CK: Creatine kinase
HK: Hexokinase
G-6-PDH: Glucose-6-Phosphate dehydrogenase
ADP: Adenosine 5' Diphosphate
ATP: Adenosine 5' Triphosphate
NADP: Nicotinamide Adenine Dinucleotide Phosphate
NADPH: Nicotinamide Adenine Dinucleotide Phosphate reduced
PMS: Phenazine Methosulfate
PMSH: Phenazine Methosulfate reduced
TNBT: Tetranitro Blue Tetrazolium

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7, 15 AND 30 ISO-CK KITS

ITEMS	PN 4111	PN 4131	PN 4137
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
Activation solution (ready to use)	1 vial, 0.75 mL	1 vial, 0.75 mL	2 vials, 0.75 mL each
Substrate Solvent (ready to use)	1 vial, 40 mL	1 vial, 40 mL	2 vials, 40 mL each
Chromogen (stock solution)	1 vial, 3 mL	1 vial, 3 mL	2 vials, 3 mL each
ISO-CK Substrate (freeze-dried)	10 vials	10 vials	20 vials
ISO CK/LD Blocking Solution (ready to use)	1 vial, 40 mL	1 vial, 40 mL	2 vials, 40 mL each
Applicators (ready to use)	1 pack of 10	1 pack of 10	2 packs of 10
Filter Papers - Thin	1 pack of 10	1 pack of 10	1 pack of 10
Filter Papers - Thick	1 pack of 10	1 pack of 10	1 pack of 10

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 ; alkaline buffer pH 8.40 ± 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 creatine kinase isoenzymes 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). (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. BUFFERED STRIPS

Preparation

Buffered sponge strips are ready to use. Each contains: alkaline buffer pH 8.3 ± 0.1 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The buffer in the strips contains 0.20 % sodium azide. Do not ingest ! Very toxic if swallowed ! 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. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water.

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. ACTIVATION SOLUTION*

Preparation

Activation solution is ready to use. It contains: buffer solution pH 6.5 ± 0.5, additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Activation solution contains ß-Mercaptoethanol. Toxic by inhalation, in contact with skin and if swallowed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If you feel unwell, seek medical advice (show the label where possible).

Use

For the treatment of serum samples before electrophoretic separations.

Storage, stability and signs of deterioration

Store activation solution refrigerated. It is stable until the expiration date indicated on the kit package or activation solution vial label.
Discard activation solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
Activation solution must be free of precipitate.

IMPORTANT : After each use, close immediately the activation solution vial to avoid its degradation due to oxidation.

NOTE : The ISO-CK Activation Solution should normally have a strong ß-mercaptoethanol odor. If this odor is fading or absent, it is a symptom of decreased or total loss of activity. The Activation Solution can be regenerated by adding pure ß-mercaptoethanol. Add 7 % by volume of the remaining amount of Activation Solution.

4. SUBSTRATE SOLVENT

Preparation

Substrate solvent is ready to use. It contains: buffer solution pH 6.70 ± 0.15, additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

For the preparation of the visualization solution, as described in paragraph 6.

Storage, stability and signs of deterioration

Store substrate solvent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or substrate solvent vial label.
Discard substrate solvent if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
Substrate solvent must be free of precipitate.

5. CHROMOGEN*

Preparation

Chromogen is ready to use. It contains: TNBT (Tetranitroblue tetrazolium) in dimethylformamide (DMF).

WARNING: Chromogen contains dimethylformamide. Harmful by inhalation. Harmful in contact with skin. Irritating to the eyes. In case of insufficient ventilation, wear suitable respiratory equipment. Do not ingest ! If ingested, consult physician immediately ! After contact with eyes or skin, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Use
For the preparation of the developing solution as described in paragraph 6.

Storage, stability and signs of deterioration
Store chromogen refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or chromogen vial label.

6. ISO-CK SUBSTRATE*

Preparation
Each substrate vial contains : Bovine albumin, Glucose, ADP (Adenosine 5' diphosphate), NADP (Nicotinamide Adenine Dinucleotide Phosphate), Hexokinase (from bakers yeast), Glucose-6-Phosphate Dehydrogenase (from bakers yeast), PMS (Phenazine Methosulfate), additives, nonhazardous at concentrations used, necessary for optimum performance.
Prepare the developing solution away from light just after the migration has started.
Gel HYDRAGEL 7 ISO CK/LD : In the substrate vial (1/2), add 2 mL substrate solvent. Close the vial and mix gently. Leave at room temperature. Just before use, add 0.15 mL chromogen and mix.
Gel HYDRAGEL ISO CK/LD 15/30 (15 samples) : In the substrate vial, add 4 mL substrate solvent. Close the vial and mix gently. Leave at room temperature. Just before use, add 0.3 mL chromogen and mix.
Gel HYDRAGEL ISO CK/LD 15/30 (30 samples) : Add 4 mL substrate solvent to each of the 2 substrate vials. Close the vials and mix gently. Leave at room temperature. Just before use, add 0.3 mL chromogen to each vial and mix.

Use
For the development of the electrophoresed creatine kinase isoenzymes.

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

7. ISO CK/LD BLOCKING SOLUTION

Preparation
The blocking solution is ready to use and contains: acetic acid, 5 % ; citric acid, 0.5 %.

Use
To stop the enzymatic reaction with the substrate after incubating the gels for specified time.

Storage, stability and signs of deterioration
The blocking solution can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the vial. Discard blocking solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

8. APPLICATORS

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

Storage, stability and signs of deterioration
Store the applicators in a dry place at room temperature or refrigerated.

9. 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.

10. THICK FILTER PAPERS

Use
Precut, single use, thick absorbent paper pads for blotting the gel before drying.

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

** NOTE: During transportation, the activation solution, chromogen and ISO-CK substrate can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.*

REAGENT REQUIRED (but supplied separately from the kit)

DESTAINING SOLUTION (optional)

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, 50 mg/dL.

Use
For rinsing the gels after scanning and the staining chamber after cleaning with wash solution.
To neutralize the acidity of the destaining solution, pour 15 mL of a 50 % solution of Sodium Hydroxide, into the empty waste container.

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

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 1215, for an alternative way of loading the sample applicators.
3. Wet Storage Chamber supplied with HYDRASYS, PN 1270.
4. Template Guide Bar SEBIA, supplied with HYDRASYS.
5. Accessory Kit for HYDRASYS ISO-CK, ISOFORMES CK, SEBIA, PN 1264.
6. Pipettes: 10 µL, 200 µL and 5 mL.
7 Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 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.
8. Quality control materials (Enzycontrôle SEBIA, PN 4790).
9. Gel holder for half gels SEBIA, PN 10043110.

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

Mix 500 µL serum sample with 5 µL activation solution, and incubate for 10 minutes at room temperature. Dilute serum samples with saline to achieve a total CK activity of about 750 IU/L when this activity is > 750 IU/L.

Samples to avoid

Do not use hemolysed samples. The erythrocyte enzymes may interfere in the reaction.
Do not run an analysis on samples that contains an inhibitor of CK enzymatic activity such as heparin, EDTA, citrate, fluorids or iodoacetate [24].

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, incubation with substrate, stopping the enzymatic reaction, blotting and final drying of the gel. The manual steps include handling samples and gels, application of reagents 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 HYDRAGEL 7 ISO CK/LD (7 samples) and HYDRAGEL ISO CK/LD 15/30 (15 samples), or two applicators for HYDRAGEL ISO CK/LD 15/30 (30 samples), on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL sample in each well. Load each applicator within 2 minutes.
   - Place the applicator(s) into the wet storage chamber with the teeth up (handle it by the plastic tooth protection frame).
   - Let the samples diffuse into the teeth for 5 minutes after the last sample application.

   See wet chamber package insert for further details.
3. Open the lid of the migration module and raise the electrode and applicator carriers.

   WARNING: Never close the lid while the carriers are raised!
4. Select «ISO-CK 7» migration program for HYDRAGEL 7 ISO CK/LD or «ISO-CK 15/30» migration program for HYDRAGEL ISO CK/LD 15/30 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 agarose gel 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 distilled or deionized water for HYDRAGEL 7 ISO CK/LD, or 200 µL for HYDRAGEL ISO CK/LD 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 from the wet chamber. Handle it by the protection frame.
   - Snap off the applicator(s) 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 applicators each into positions No. 3 and 8 on the carrier.

   IMPORTANT: The numbers printed on the applicator must face the operator (Fig. 4).
9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow «START» key on the left side of the keyboard.

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

MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

- The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out under 10 W constant for HYDRAGEL 7 ISO-CK, or 20 W constant for HYDRAGEL ISO-CK 15/30, at 20 °C controlled by Peltier effect until 27 Vh accumulated (for about 6 minutes).
- The electrode carrier rises to disconnect the electrodes.
- At the end of the migration step, the ventilation starts automatically for 2 minutes. A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following flashing message is displayed on the screen: « ⇩ SUBSTRATE».

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

II. PREPARATION FOR INCUBATION WITH ISO-CK SUBSTRATE

1. After the migration, open the lid. The following message stops flashing : « ⇩ SUBSTRATE ».
2. Remove the sample applicator(s) and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Clean the electrodes by wiping them carefully with a soft wet tissue.
   - Leave the gel in place in the migration module.
4. Set up the corresponding reagent application CK template, template R1, R2 or R4 for 7, 15 or 30 samples analysis, respectively as follows (Fig. 5):
   - Position the application template guide on the anchoring clips (the guide can be left on the HYDRASYS at all times).
   - Hold the flap on the template and put the notches in the guide marks.
   - Lower the template onto the gel.
5. After having mixed chromogen and substrate in the vial, apply immediately 1.8 mL of ISO-CK substrate solution for HYDRAGEL 7 ISO-CK, 4 mL for HYDRAGEL ISO-CK 15/30 (15 samples) or 7.5 mL for HYDRAGEL ISO-CK 15/30 (30 samples), as follows (Fig. 6):
   - 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.
6. Close the HYDRASYS cover.
7. Start immediately the incubation procedure by pressing the green arrow «START» key on the left side of the keyboard. The following message is displayed on the screen: «INCUBATION».

INCUBATION - DESCRIPTION OF AUTOMATIC STEPS

- Incubation at 37 °C controlled by Peltier effect, for 30 minutes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following flashing message is displayed on the screen: « ⇧ SUBSTRATE ⇩ BLOCKING ».

NOTE: During the incubation step, the cover of the migration module remains locked.

III. SUBSTRATE ELIMINATION AND APPLICATION OF BLOCKING SOLUTION

1. Open the cover. The following flashing message stops flashing: « ⇧ SUBSTRATE ⇩ BLOCKING».
2. Remove the remaining substrate solution :
   - Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig. 6).
   - Carefully and progressively withdraw the reagent.
3. Pipet 2 mL of blocking solution for HYDRAGEL 7 ISO-CK, 4 mL for HYDRAGEL ISO-CK 15/30 (15 samples) or 8 mL for HYDRAGEL ISO-CK 15/30 (30 samples) into the template (Fig. 6). Do not introduce air bubbles.
4. Close the HYDRASYS cover.
5. Start the run by pressing the «START» key (green arrow on the left side of the keyboard).
   The following message is displayed on the screen: «BLOCKING».

BLOCKING - DESCRIPTION OF AUTOMATIC STEPS

- Incubation at 37 °C, for 10 minutes.
- A beep sounds and the cover unlocks. The following flashing message is displayed on the screen: « ⇧ BLOCKING ⇩ PAPER».

NOTE: During the incubation step, the cover of the migration module remains locked.

IV. ELIMINATION OF BLOCKING SOLUTION AND FILTER PAPER APPLICATION

1. Open the cover.
2. Eliminate the blocking solution with a pipet (see SUBSTRATE ELIMINATION).
3. Remove the template:
   - Grasp the flap of the template.
   - Raise the template and remove it.
4. Apply one thick filter paper on the gel:
   - 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.
5. Close the HYDRASYS cover.
6. Start the blotting sequence by pressing the «START» key (green arrow on the left side of the keyboard). The following message is displayed on the screen: «BLOTTING».
7. 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.

BLOTTING - DESCRIPTION OF AUTOMATIC STEPS

- Blotting at 37 °C controlled by Peltier effect, for 3 minutes.
- A beep sounds until the operator intervenes. The following flashing message is displayed on the screen: « ⇧ PAPER».

NOTE: During the blotting step, the cover of the migration module remains locked.

V. DRYING OF THE GEL

1. Open the cover.
2. Remove the filter paper and leave the gel in place.
3. Close the cover of HYDRASYS.
4. Start the drying step by pressing the «START» key (green arrow on the left side of the keyboard). The following message is displayed on the screen : «DRYING».

DRYING - DESCRIPTION OF AUTOMATIC STEPS

- Drying of the gel at 60 °C, for 2 minutes.
- A beep sounds and the cover unlocks. The plate temperature remains at 50 °C until the lid is opened.

NOTE: During the drying step, the cover of the migration module remains locked.

VI. GEL SCANNING

1. Open the cover.
2. Remove the dried gel for densitometry.
3. Clean the back side (the plastic support side) of the dried film with a damp soft paper.
4. If needed, take off any lint from the gel side with soft paper.
5. 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.

6. The temperature of the plate decreases to 20 °C in less than 5 minutes. When 20 °C is reached, a new migration run can be started.
7. Position the sample applicator and electrode carriers in place.
8. Wipe the temperature control plate with a soft wet tissue.

VII. WASH OF THE GEL

After scanning, the gel can be rinsing for a better preservation of the electrophoregrams (to prevent development of background stain).

1. Remove the gel plate for further processing.
2. Open the gel holder. Lay it flat and position the 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. 7).
3. Place the gel holder into the gel processing / staining module.
   IMPORTANT: Before starting the gel processing / staining program, check the following :
   - the wash solution container contains at least 400 mL of 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.
4. Select «WASHING ISOENZYMES» washing program from the instrument menu and start the run by pressing the «START» key (green arrow on the right side of the keyboard).
   During washing 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).
5. Remove the gel holder from the compartment ; open the clips and remove the dried gel. If needed, clean the back side (the plastic support side) of the dry film with a wet tissue paper.

RESULTS

Quality Control

It is advised to include an assayed control serum (e.g., Enzycontrole SEBIA, PN 4790) into each run of samples.

Values[19]

If the sample does not show any MB or BB fraction, it is not necessary to scan.
Human serum may show three fractions:

- the MM fraction, predominant in normal human serum, with a very low mobility (gamma zone),
- the MB fraction with an intermediate mobility (beta zone),
- the BB fraction with the highest mobility (alpha-1 zone).

Normal values:

CK MM : 97 to 100 %
CK MB : 0 to 3 % if CK activity is comprised between 15 and 500 IU/L
CK MB : 0 to 4 % if CK activity is higher than 500 IU/L
CK BB : 0 %
It is recommended each laboratory establishes its own normal values.

Interpretation

- MB fraction increases 4 to 8 hours after myocardial infarction.
- BB fraction is increased primarily in some disorders and trauma of the nervous system.

- Total creatine phosphokinase (mainly MM) is increased in the following cases:
  - surgery trauma
  - intensive physical exercises
  - muscular dystrophy
  - myopathy
  - polymyositis
  - hypothyroidism
  - drugs, intramuscular injections.
- The macro CK (type 1) is a complex between CK and immunoglobulins. It is detectable between MM and MB fractions (Fig. 8).
- The mitochondrial CK (type 2) is detectable on the cathodic side of the MM fraction (Fig. 8).

Troubleshooting

Call Technical Service of the supplier when the test fails to perform while the instructions for the preparation and storage of materials, and for the procedure were carefully followed.
Kit reagent Safety Data Sheets and 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 on HYDRAGEL ISO CK/LD 15/30 gels from two lots. The samples were : A - normal serum with only MM ; B - elevated MB ; C SEBIA Enzycontrole. Each sample was applied in all 15 tracks of a single gel from each lot. The electrophoregrams were evaluated by densitometry.
The following table shows the means, SD, CV for each CK fraction (sample B and C) for each gel of the two lots and for the pooled data from the two lots (the 15 tracks from each gel/lot were combined for each sample). Sample A had only MM fraction which was detected in all tracks on each gel from the two lots.

PARAMETER	MM	MB	BB
Sample B : lot no. 1 / lot no. 2 / pooled data			
MEAN (%)	92.9 / 93.5 / 93.2	7.1 / 6.5 / 6.8	/
SD	0.4 / 0.2 / 0.3	0.4 / 0.2 / 0.3	/
CV (%)	0.4 / 0.2 / 0.3	5.6 / 3.4 / 4.5	/
Sample C : lot no. 1 / lot no. 2 / pooled data			
MEAN (%)	49.0 / 48.9 / 48.9	17.5 / 17.7 / 17.6	33.5 / 33.4 / 33.5
SD	0.5 / 0.4 / 0.4	0.4 / 0.3 / 0.4	0.4 / 0.4 / 0.4
CV (%)	1.0 / 0.8 / 0.9	2.3 / 1.8 / 2.1	1.2 / 1.3 / 1.3

Between gels and lot-to-lot reproducibility

Fifteen different serum samples were run each on 10 HYDRAGEL ISO CK/LD 15/30 gels from 3 lots. The means CV, SD and CV (n = 10) were calculated for each serum sample and each CK 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 (n = 15).

CK FRACTION	SD	CV (%)	MEAN CV (%)
MM	0.1 - 0.8	0.1 - 0.9	0.4
MB	0.1 - 0.8	2.7 - 14.7	6.0
BB	0.1 - 0.4	1.2 - 7.3	4.2

Accuracy - Comparative study

Seventy (70) different serum samples (normal and pathological) were analyzed using SEBIA's HYDRAGEL 15 & 30 ISO-CK kit and another, commercially available test for electrophoretic determination of CK isoenzymes, with fluorescence. There was a perfect agreement between the two tests in visual detection of the CK isoenzymes. The results of linear regression analysis of the densitometric values of MB fraction (n = 70) are tabulated below.

PARAMETER	Correlation coefficient	y-intercept	Slope	Range of % values (SEBIA's test)
MB fraction	0.950	-0.427	0.869	0.8 - 21.7

y = SEBIA values

Sensitivity

A pathological serum sample with a MB fraction (activity: 40 IU/L) was serially diluted and the dilutions electrophoresed on HYDRAGEL ISO CK/LD 15/30 gel. After densitometry, the lowest detected activity of a MB fraction was 2,5 IU/L.

Linearity

Serial dilutions of a sample containing the MM and MB fractions were tested, from total CK activity of 750 IU/L to 20 IU/L. The system was linear in the entire range studied.

BIBLIOGRAPHY

1. Abbott LB, Van Lente F, 1985. Procedure for characterization of creatine kinase variants on agarose electrophoretograms. *Clin Chem*, 31, 3 : 445.
2. Anido VA, Conn RB, Mengoli HF, Anido G, 1974. Diagnostic efficacy of myocardial creatine phosphokinase using polyacrylamide disk gel electrophoresis. *Am J Clin Pathol*, 61 : 599.
3. Cawley LP, 1969. Electrophoresis and immunoelectrophoresis. Little, Brown and Company, Boston.
4. Doran GR, Wilkinson JH, 1975. The origin of elevated activities of creatine kinase and other enzymes in the sera of patients with myxedema. *Clin Chim Acta*, 62 : 203.
5. Dubo H, Park DC, Pennington RJT, Kalbag RM, Walton JN, 1967. Serum-creatine-kinase in cases of stroke, head injury and meningitis. *Lancet*, 2 : 743.
6. Jockers-Wretou E, Pfleiderer G, 1975. Quantitation of creatine kinase isoenzymes in human tissues and sera by an immunological method. *Clin Chim Acta*, 58 : 223.
7. Kachmar JF, Moss DW: Enzymes. In Fundamentals of Clinical Chemistry. NW Tietz, Editor, Saunders, Philadelphia, 1976, pp 652-660.
8. Keffer JH, 1996. Myocardial markers of injury. *Clin Chem*, 105, 3 : 305.
9. King JO, Zapf P, 1972. A review of the value of creatine phosphokinase estimations in clinical medicine. *Med J Aust*, 1 : 699.
10. Konttinen A, Somer H, 1973. Specificity of serum creatine kinase isoenzymes in diagnosis of acute myocardial infarction. *Br Med J*, 1, 386.
11. Konttinen A, Somer H, Auvinen S, 1974. Serum enzymes and isoenzymes. Extrapulmonary sources in acute pulmonary embolism. *Arch Intern Med*, 133 : 243.
12. LaFair JS, Myerson RM, 1968. Alcoholic myopathy. *Arch Intern Med*, 122 : 417.
13. Mair J, Morandell D, Genser N, Lechleitner P, Dienstl F, Puschendorf B, 1995. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. *Clin Chem*, 41, 9 : 1266.
14. Morin LG, 1977. Creatine kinase : stability, inactivation, reactivation. *Clin Chem*, 23, 4 : 646
15. Nealon DA, Henderson AR, 1975. Measurement of brain-specific creatine kinase isoenzyme activity in serum. *Clin chem*, 21 : 1663.
16. Rao PS, Mueller HS, 1976. Evaluation of the chemical activation procedure (Rao's method) for the measurement of the MB isoenzyme of creatine kinase. *Clin Chem*, 22 : 932.
17. Rock RC, Dreskin R, Kickler T, Wimsatt T, 1975. Creatine kinase isoenzymes in serum of patients with Reye's syndrome. *Clin Chim Acta*, 62 : 159.
18. Roe CR, Limbird LE, Wagner GS, Nerenberg ST, 1972. Combined isoenzyme analysis in the diagnosis of myocardial injury : Application of electrophoretic methods for the detection and quantitation of the creatine phosphokinase MB isoenzyme. *J Lab Clin Med*, 80 : 577.
19. Thompson WG, Mahr RG, Yohannan WS, Pincus MR, 1988. Use of creatine kinase MB isoenzyme for diagnosing Myocardial infarction when total creatine kinase is high. *Clin Chem*, 34, 11 : 2208.
20. Vassella F, Richterich R, Rossi E, 1965. The diagnostic value of serum creatine kinase in neuromuscular and muscular disease. *Pediatrics*, 35 : 322.
21. Vincent WR, Rapaport E, 1965. Serum creatine phosphokinase in the diagnosis of acute myocardial infarction. *Am J Cardio*l, 15 : 17.
22. Weime RJ, 1965. Agar Gel Electrophoresis, Elsevier, Amsterdam.
23. Wolk PL, Kearns T, Nuehoff J, Lauridson J, 1974. Identification of CPK isoenzyme MB in myocardial infarction. *Lab Med*, 5 : 48.
24. Young DS, Pestaner LC, Gibberman V. *Clin Chem*, 21 : 1D (1975).
25. Zimmerman HJ, Henry JB: Clinical Enzymology. In Clinical Diagnosis and Management by Laboratory Methods, 16th ed., JB Henry, Editor, Saunders, Philadelphia, 1979, pp 365-368.
26. Zsigmond EK, Starkweather WH, 1963. Abnormal serum and muscle creatine phosphokinase (CPK) isoenzyme pattern in a family with malignant hyperthermia. *Anaesthesis*, 22 : 16.
27. Zsigmond EK, Starkweather WH, Duboff GS, Flynn KA, 1972. Elevated serum creatine phosphokinase activity in a family with malignant hyperpyrexia. *Anesth analg*, 51 : 827.

SCHÉMAS / FIGURES

Figure 1

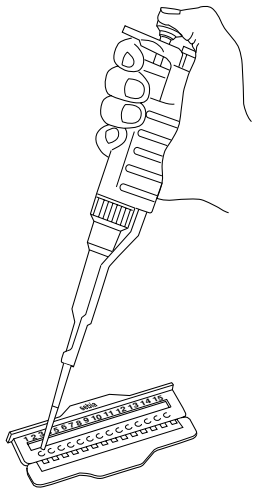


Figure 2

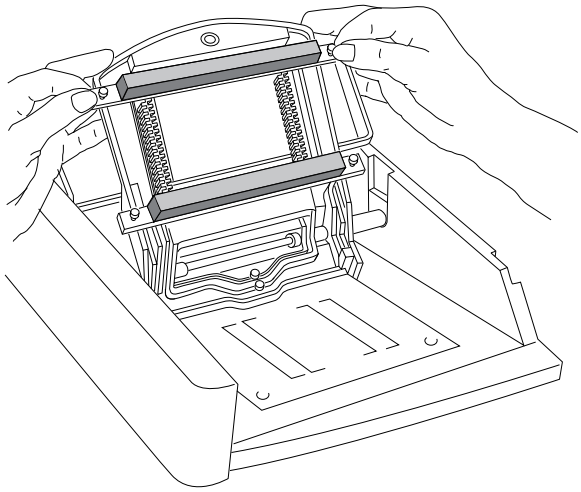


Figure 3

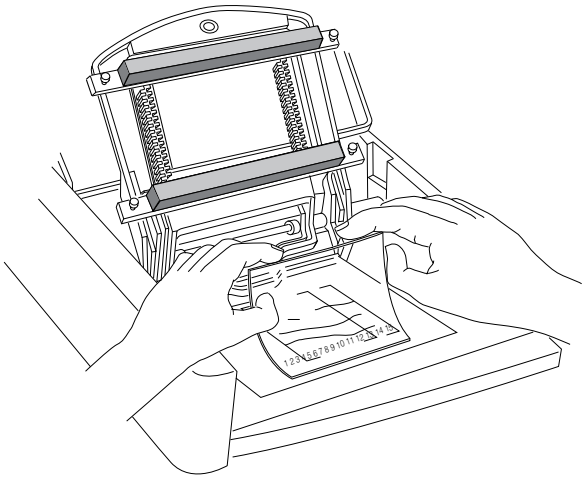


Figure 4

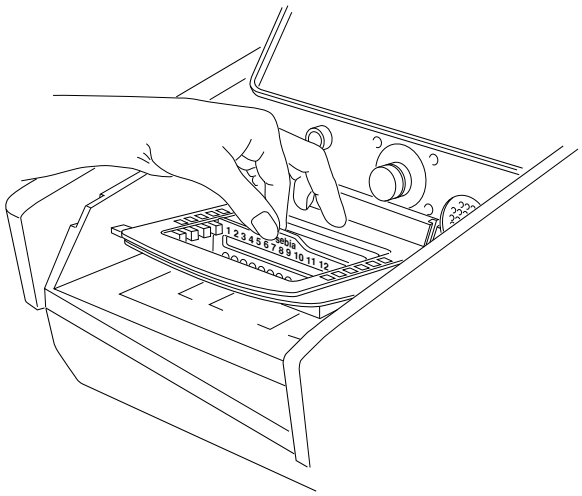


Figure 5

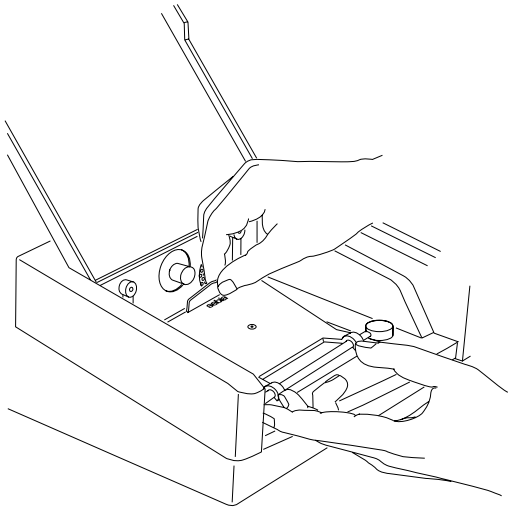


Figure 6

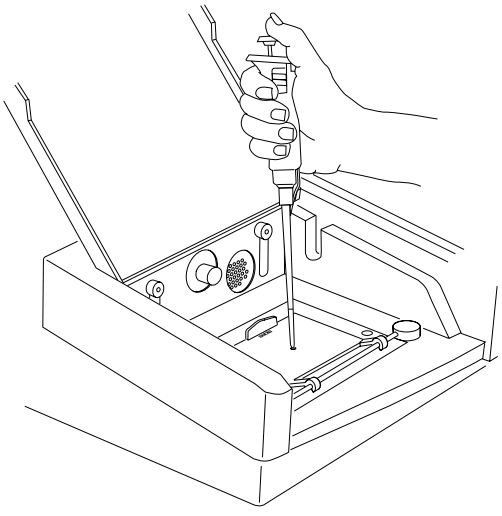


Figure 7

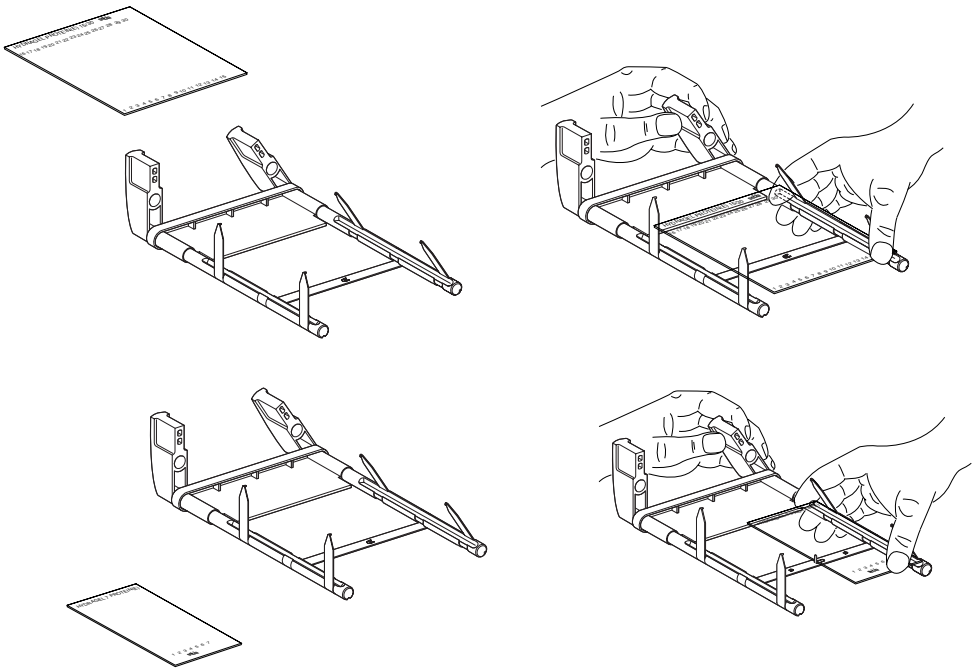
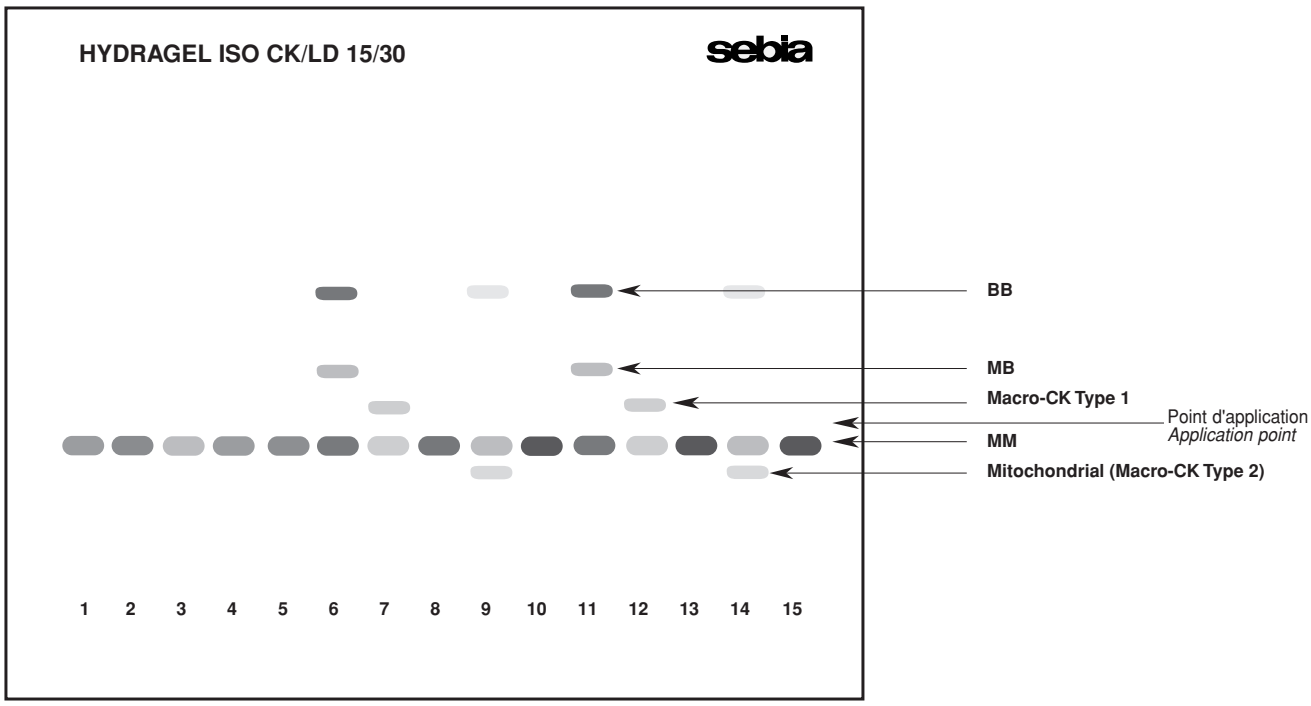


Figure 8

Identification des fractions
Fraction identification



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.