HYDRAGEL LP CIII
Ref. 4056
INTENDED USE

The HYDRAGEL LP CIII kit is designed for the quantification of total apo CIII and apo CIII present in the particles without apo B (apo CIII LP non B) in human serum by electroimmunodiffusion on mildly alkaline, buffered (pH 7.6) agarose gels. The gels contain anti-apo CIII monospecific antibody. After migration, the resulting rockets are stained with acid violet. The excess stain is removed with an acidic solution.

A calibration plot is constructed from assayed values obtained on a calibrated apo CIII standard serum. Heights of the patient’s rockets then provide serum levels of apo CIII as follows: difference between the two rockets (treated sample in order to eliminate particles containing apo B, and native sample) yields concentration of apo CIII in the particles containing apo B (apo CIII LP B or LP B: CIII).

Each agarose gel in the HYDRAGEL LP CIII kit is intended to run 7 samples.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

The dyslipoproteinemias are one of the best predictors of cardiovascular diseases. The relations between the circulating lipids and atherosclerosis are now well established. There are atherogeneous lipoproteins, favouring the arterial cholesterol accumulation, and non atherogeneous lipoproteins that can remove cholesterol from the tissues to the liver which catabolizes and excretes it.

Many lipoprotein classifications and quantifying methods are today available. Advance in molecular biology enriches continually the wide biological test range.

Lately, immunological methods have been developed that allow lipoproteins to be classified on the basis of their apolipoprotein composition:

- Particles consisting of lipids and one single apolipoprotein are called «LP» followed by the apolipoprotein determining symbol (e.g., LP B, LP Al).
- Particles consisting of lipids and several apolipoproteins are called «LP» followed by the symbols in the order of decreasing apolipoprotein concentration order (e.g., LP Al: AII, LP B: E, LP B: CIII: E).

Apolipoprotein CIII is often associated with other apolipoproteins. It is found both in atherogeneous lipoproteins VLDL, LDL and IDL mainly combined with apo B, apo CII and apo E, and in non atherogeneous lipoproteins HDL, principaly combined with apo AI, apo AII, apo CII and apo E. Apo CIII LP non B / apo CIII LP B ratio is closely connected to the lipolytic activity and stabilization or decline of atherosclerotic plaque (Blankenhorn, Circulation, 1990, 81, 470).

In order to characterize these two types of particles whose functions are completely opposite, it is necessary to perform a differential quantification.

- Firstly, determination of total apo CIII concentration on native serum.
- Secondly, determination of apo CIII LP non B concentration on treated serum. Anti-apo B antibody added to the native sample precipitate all classes of apo B containing particles, including the complex particles e.g., LP B: CIII, LP B: CIII: E.

Difference between the rockets resulting from native and treated samples yields concentration of particles consisting of both apo B and apo CIII.

The application of the electrodiffusion method to ready-to-use gels permits the quantification of LP CIII particles in daily practice.

ABBREVIATIONS:

VLDL: Very Low Density Lipoproteins

LDL: Low Density Lipoproteins

IDL: Intermediary Density Lipoproteins

HDL: High Density Lipoproteins

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL LP CIII KIT

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4056</th>
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</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
</tr>
<tr>
<td>TGM Buffer (stock solution)</td>
<td>3 vials, 100 mL each</td>
</tr>
<tr>
<td>Acid Violet Stain (stock solution)</td>
<td>1 vial, 75 mL</td>
</tr>
<tr>
<td>Destaining Solution (stock solution)</td>
<td>1 vial, 100 mL</td>
</tr>
<tr>
<td>Standard Serum (freeze-dried)</td>
<td>10 vials</td>
</tr>
<tr>
<td>Anti-apo B Immunoglobulins (ready to use)</td>
<td>1 vial, 4 mL</td>
</tr>
<tr>
<td>Anti-apo B Diluent (ready to use)</td>
<td>1 vial, 50 mL</td>
</tr>
<tr>
<td>Filter Papers - Thin</td>
<td>2 packs of 10 each</td>
</tr>
<tr>
<td>Filter Papers - Thick</td>
<td>4 packs of 10 each</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, 0.9 g/dL; alkaline buffer pH 7.6 ± 0.1; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for electroimmunodiffusion.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging and refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards). Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.

DO NOT FREEZE.
Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. TGM 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: tris-glycine-MES (Morpholino Ethane Sulfonate) buffer pH 7.8 ± 0.3; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Electrophoresis buffer.

Storage, stability and signs of deterioration
Store stock buffer solution at room temperature or refrigerated. It is stable for several years, 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. ACID VIOLET STAIN
Preparation
Vial of the stock acid violet stain to be diluted up to 300 mL with distilled or deionized water.

After dilution, the working stain solution contains: acid solution pH \( \approx 2 \); acid violet, 0.2 g/dL; ethylene-glycol, 3.25 %; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Harmful if swallowed.

Use
For staining gels after electroimmunodiffusion.

Storage, stability and signs of deterioration
Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicated on the kit package or stain vial labels. Working stain solution is stable for 6 months.

4. DESTAINING SOLUTION
Preparation
The vial of stock destaining solution to be diluted up to 10 liters with distilled or deionized water. It is convenient to prepare a 1/100 dilution of only a small aliquot of the stock solution, e.g., dilute 10 mL stock solution up to 1 liter.

After dilution, the working destaining solution contains: citric acid, 0.5 g/dL.

Use
For destaining, that is removal of excess and background stain from the gels.

Storage, stability and signs of deterioration
Store stock destaining solution at room temperature or refrigerated. Stock solution is stable until the expiration date indicated on the kit package or destaining solution vial labels. Diluted destaining solution is stable for one month at room temperature in a closed bottle.

Discard diluted destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination. Do not add any sodium azide.

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.

5. STANDARD SERUM*
LP CIII standard serum is made from a human serum pool, stabilized by a particular process and freeze-dried.

WARNING: Any test being able to prove absence of HIV, B and C hepatitis viruses or any other infectious agent, this standard serum has to be handled according to the usual precautions in order to prevent contamination.

Preparation
Reconstitute one vial of lyophilized serum with 0.5 mL distilled water. Wait for 30 minutes at room temperature (15 to 30 °C). Mix gently, avoiding foam development.

Dilute the standard as follows and vortex.

<table>
<thead>
<tr>
<th>Points</th>
<th>Saline (µL)</th>
<th>Standard (µL)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>870</td>
<td>30</td>
<td>Standard x 0.5</td>
</tr>
<tr>
<td>2</td>
<td>840</td>
<td>60</td>
<td>Standard x 1</td>
</tr>
<tr>
<td>3</td>
<td>780</td>
<td>120</td>
<td>Standard x 2</td>
</tr>
<tr>
<td>4</td>
<td>720</td>
<td>180</td>
<td>Standard x 3</td>
</tr>
</tbody>
</table>

Use
For the calibration.

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

Store reconstituted standard serum refrigerated up to 5 days.

Store calibration solutions refrigerated. They are stable for up to one day.
6. ANTI-APO B IMMUNOGLOBULINS*

Preparation
Anti-apo B immunoglobulins are ready to use. They are total mammalian anti-human apo B immunoglobulins.

Use
For sample treatment, that is precipitation of all particles containing apo B.

Storage, stability and signs of deterioration
Store immunoglobulins refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or immunoglobulins vial labels. Discard immunoglobulins if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

7. ANTI-APO B DILUENT*

Preparation
Anti-apo B diluent is ready to use.

Use
For the dilution of the samples treated by the anti-apo B immunoglobulins.

Storage, stability and signs of deterioration
Store diluent refrigerated. It is stable until the expiration date indicated on the kit package or diluent vial labels. Diluent must be free of precipitate.

8. FILTER PAPERS - THIN

Use
Precut, single use absorbent paper pads for blotting remaining proteins off the gel.

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

9. FILTER PAPERS - THICK

Use
Precut, single use absorbent paper pads for blotting remaining proteins off the gel.

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

* NOTE: During transportation, the gels, standard serum, anti-apo B immunoglobulins and anti-apo B diluent 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
For standard serum and native sample dilutions. To eliminate remaining proteins from the gels.

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. Electrophoresis Chamber: K 20 SEBIA, PN 1400.
3. Tanks and Gel Holders for processing of HYDRAGEL gel plates: HYDRAGEL K20 Accessory Kit SEBIA, PN 1420.
4. Pipettes: 5 µL, 200 µL and 1000 µL.
5. Incubator-Dryer for drying agarose gel plates: IS 80 SEBIA, PN 1430.
6. EID Scanning System SEBIA, PN 1100.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh serum samples are recommended for analysis. Sera must be collected according to established procedures used in clinical laboratory testing. If needed, store sera at 2 to 8 °C for up to one week. Keep samples frozen for longer storage periods; frozen samples are stable for at least one month.

Sample preparation
- Native samples: add 60 µL serum and 840 µL saline.
- Anti-apo B treatment: in a test tube, add in the following order, 60 µL anti-apo B immunoglobulins, 60 µL serum and 780 µL anti-apo B diluent. Vortex and incubate for 5 to 10 minutes at room temperature. Centrifuge at 3000 rpm for 10 minutes. Take the supernatant (which will be analyzed).
PROCEDURE

I. MIGRATION STEP

1. Unpack the gel. The gel can be conveniently placed on the top of the closed gel box.
2. Apply 5 µL previously diluted native or treated sample or diluted standard in each well. Wipe the pipette tip before each application. Do not apply sample or standard in the first and last wells, in order to avoid edge effect. Fill them with 5 µL saline.
3. Let the samples diffuse into the gel for 20 minutes.
4. When using SEBIA K20 chamber, place the HYDRAGEL on the bridge with the gel side facing down; the bridge must be outside the chamber to avoid buffer to get into the wells. Then place the bridge carefully in the chamber, samples on the cathodic side. The gel dips about 1 cm into the buffer on each side.

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

<table>
<thead>
<tr>
<th>MIGRATION CONDITIONS</th>
<th>SEBIA K20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of buffer per compartment</td>
<td>150 mL</td>
</tr>
<tr>
<td>Total buffer volume</td>
<td>300 mL</td>
</tr>
<tr>
<td>Migration time</td>
<td>4 h</td>
</tr>
<tr>
<td>Constant voltage</td>
<td>50 V</td>
</tr>
<tr>
<td>Initial current (per gel)</td>
<td>17 ± 3 mA</td>
</tr>
<tr>
<td>Migration time</td>
<td>2 h</td>
</tr>
<tr>
<td>Constant current (per gel)</td>
<td>15 mA</td>
</tr>
<tr>
<td>Initial voltage</td>
<td>45 ± 3 V</td>
</tr>
</tbody>
</table>

6. After migration, unplug the chamber and remove the gel plate. Lay it on a flat surface.

II. REMOVAL OF THE REMAINING PROTEINS

1. Apply one thin filter paper previously soaked with saline on the gel. Add two layers of thick filter paper and place a weight of about 1 to 1.5 kg on the top of the stack for 20 minutes. Make sure that the weight is evenly distributed. When several gels are processed at the same time, they can be formed into one stack.
2. Remove the filter papers. Wash the gel vertically in saline for 60 minutes minimum.
3. Apply one thin filter paper previously soaked with saline on the gel. Add two layers of thick filter paper and place a weight of about 1 to 1.5 kg on the top of the stack for 10 minutes.
4. Remove the filter papers. Dry the gel completely in the incubator-dryer at 80 °C.

III. STAINING AND DESTAINING STEPS

1. Immerse the dried and cooled gel vertically in the staining solution for 5 minutes.
2. Destain in three successive baths of destaining solution until the background is completely colorless and clear.
3. Remove excess liquid on the gel surface with a soft paper and dry the gel in a 80 °C air stream. If necessary, clean the back side (support side) with a damp soft paper.

IV. READING

1. Measure the rockets.
2. Construct the calibration curve, plotting apo CIII standard concentrations on the X-axis and the corresponding rocket heights on the Y-axis.

RESULTS

Quality Control

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

Values

For the samples, measure the heights of the two rockets: one obtained on the native serum (total apo CIII) and the other obtained after anti-apo B treatment (apo CIII LP non B). Report them on the Y-axis of the calibration curve and read the corresponding apo CIII concentrations.

The difference between the two concentrations obtained on the same serum, gives directly the concentration of all particles containing apo B and apo CIII (apo CIII LP B or LP B: CIII).

Normal values obtained on 800 normolipemic subjects:

Total apo CIII : 1.6 to 4.5 mg/dL
Apo CIII LP non B : 0.5 to 3.5 mg/dL
Apo CIII LP B or LP B: CIII : ≤ 2.3 mg/dL

It is recommended each laboratory establishes its own normal values.

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.

BIBLIOGRAPHY


