IVD CE

Instructions for Use (IFU)
Agarose Gel Lipoprotein Electrophoresis Kit
REF.: LE

This document represents the full Instructions for Use. A short paper (version) containing essential information for safe use may be supplied with the product.

Regulatory Framework: Regulation (EU) 2017/746 on "in vitro diagnostic medical devices" (IVDR) Annex I, Chapter III §20.4/ Annex II §2.
CE Mark: In conformity with Annex V of the IVDR 2017/746.

Catalogue Number (REF)	UDI-DI
LE10	5213012290405
MLE	5213012290276

1. Intended Purpose
The Hellabio Lipoprotein Electrophoresis Kit is intended for the qualitative separation and screening of human serum lipoprotein fractions (Chylomicrons, β-Lp, pre-β-Lp, and α-Lp) based on their electrophoretic mobility in agarose gel. The kit is used as a screening tool for the clinical evaluation of dyslipidemias and the identification of atherogenic risk factors, such as **Lipoprotein(a) [Lp(a)]**.
For in vitro diagnostic use only, by qualified laboratory professionals.

2. Summary and Principle of the Method
Lipoproteins are circulating complexes of lipids and proteins. At pH 8.6, lipoproteins carry a negative charge and migrate toward the anode. Agarose gel electrophoresis separates these complexes into distinct zones. Following electrophoresis, the lipoproteins are visualized using a lipid-specific stain (Fat Red 7B or Sudan Black). Evaluation is performed visually for pattern abnormalities or by densitometry for relative quantification. Any densitometric evaluation provides relative pattern information only and is not intended for absolute quantitative measurement or standalone clinical decision-making.

3. Reagents and Materials Provided

Component	Content LE10	Content MLE
Agarose Gels	10	12
Electrophoresis Buffer (50X Concentrate)	20 ml	20 ml
Staining Solution (Stock solution)	60 ml	33 ml
NaOH solution	8 ml	5.5 ml
Gel Blotter Strips	20	24
Sample Templates	10	12
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4. Additional Materials Required but Not Provided

- Electrophoresis tank and power supply (capable of providing a constant 100 -220 Volts)
- Staining and destaining baths
- Precision pipettes
- Densitometer or Gel Analyzer (e.g., HellabioScan) with a 520-600 nm filter
- Hot air oven or dryer (up to 90°C)
- Distilled or deionized water

5. Warnings and Precautions

- **For *in vitro* diagnostic use only.**
- This device is intended for professional use only by qualified laboratory personnel.
- All human source materials (specimens, controls) should be handled as potentially infectious. Follow standard biosafety precautions (e.g., wear protective gloves, lab coats, and eye protection).
- Do not use any component of the kit beyond its expiration date.
- Do not mix reagents from different kit lots.
- Avoid using gels that appear dry, contaminated, or physically damaged.
- Avoid using grossly hemolytic, icteric, or lipemic samples as they may cause erroneous results.
- **Reagent Warning:** The Electrophoresis Buffer and Protein Diluent Solution contain **sodium azide (NaN_3) < 0.1%** as a preservative.
  - **H302:** Harmful if swallowed.
  - **EUH032:** Contact with acids liberates very toxic gas.
  - Avoid contact with skin and eyes. In case of contact, rinse immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Dispose of waste by flushing with a large volume of water.
- Refer to the **Safety Data Sheet (SDS)**, available at www.hellabio.com, for detailed information on chemical safety.
- **Reporting Serious Incidents:** Any serious incident that has occurred in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

6. Reagents Preparation, Storage, and Stability

- **Storage:** Store the entire kit horizontally at **15-25°C**. Do not freeze.
- **Stability:** All components are stable until the expiration date indicated on the label when stored as directed.

Reagents	Preparation		Storage & Stability of Working Solution
Agarose Gels	Ready to use. Carefully remove from packaging just before use.		Store at 15 - 25°C on horizontal position until the expiration date indicated on the kit.
Electrophoresis Buffer	Dilute the 50X concentrate as instructed on the bottle with distilled water.		Store at room temperature (15-25°C) for up to 2 months.
Staining Solution	**Fat Red**	**LE10**: Dilute 5ml of stock solution in a clean tube with 700µl from 0.2M NaOH solution under continuing mixing.	Store in a closed flask at room temperature (15-25°C) until the expiration date of the kit.

Reagents	Preparation		Storage & Stability of Working Solution
		MLE: Dilute 2ml of stock solution in a clean tube with 300µl from 0.2M NaOH solution under continuing mixing.	
	Sudan Black	**LE10:** Dilute 5ml propanol with 160µl stock solution and 6ml dest. water.	Store in a closed flask at room temperature (15-25°C) until the expiration date of the kit.
		MLE: Dilute 2.5ml propanol with 80µl stock solution and 3ml dest. water.	
		LE10: Dilute 6ml ethanol with 75µl stock solution and 5ml dest. water.	
		MLE: Dilute 3ml ethanol with 40µl stock solution and 2.5ml dest. water.	
NaOH Solution for Fat Red Staining Solution	Ready to use		Store at room temperature (15-25°C).
Destaining Solution	**For Fat Red**	60% v/v methanol (not included in the kit).	Store at room temperature (15-25°C).
	For Sudan Black	40% v/v propanol or 60% v/v ethanol (not included in the kit).	

- **Note:** If crystals form in the concentrated buffers, warm the vial in a water bath until they dissolve.

7. Sample Collection and Preparation

- **Serum:** Fresh serum collected after 12-14 hours fast is mandatory.
- **Stability:** Samples are stable for 3 days at 2-8°C. Do not freeze, as freezing alters lipoprotein patterns (especially VLDL and Chylomicrons).

8. Procedure

1. Before the electrophoresis process prepare the staining and destaining solutions according to the preparation instructions. Use undiluted serum or plasma as a sample.
2. Fill the electrophoresis chamber with adequate volume (it depends on the chamber volume) of electrophoresis buffer.
3. Take the agarose gel out of its packaging, uncover it from the plastic plate and put it on the backside of the plate in horizontal position. Keep the plastic tray (**for staining**).
4. Blot the gel for 5'' with a gel blotter strip on the sample application zone.
5. Place the sample template on the application zone. Rub the template with the forefinger so that it gets in contact with the gel surface.
6. Using a 5 µl pipette, apply:
    - **5 µl** of the samples across each corresponding slit **for LE10** and let them absorb into the gel for:
        - **2 minutes for Fat Red**
        - **5 minutes for Sudan Black**.

- **3 µl** of the samples across each corresponding slit **for MLE** and let them absorb into the gel for:
  - **2 minutes for Fat Red**
  - **5 minutes for Sudan Black**.

The application of the samples should be done as quickly as possible. The application slits should not be allowed to dry.

7. Blot the excess sample with a gel blotter strip, gently remove both the sample template and the gel blotter strip and discard them.
8. Place the gel into the tank in the right charge position, connect the tank to the power supply and run:
   - **for LE10 20 minutes/ 100 Volts.**
   - **for MLE 18 minutes/ 100 Volts.**
9. Following the electrophoresis, switch off the power supply, put the gel on the plastic tray and dry it with hot air (less than 85°C).
10. Place the plastic tray with the gel in a horizontal position and stain it according to the preparation instructions. Fill the plastic tray with the corresponding staining solution and stain the gel for **4 minutes**.
11. After the staining procedure, decolonise the gel with destaining solution **two times for 2 minutes each**.
12. Dry again the gel and interpret the results visually or by HellabioScan or a densitometer (520 nm).

9. Interpretation of Results & Clinical Significance

- **Normal Profile:** Visualized as α-Lp (HDL), pre-β-Lp (VLDL), and β-Lp (LDL) bands.
- **Lipoprotein(a) [Lp(a)] Detection:** When present at detectable concentrations, Lp(a) typically migrates between the pre-β (VLDL) and β (LDL) zones. Its presence is a significant independent risk factor for coronary heart disease.
- **Small Dense LDL (sdLDL):** Variations in the mobility or thickness of the β-Lp band may indicate the presence of atherogenic small dense LDL particles (Pattern B).

10. Limitations and Interferences

- **Screening Method:** This method is a qualitative screening tool. For definitive diagnosis and risk assessment, results must be correlated with quantitative assays (e.g., enzymatic lipid panels, nephelometric Lp(a) measurement).
- **Therapeutic Interference:** Modern lipid-lowering therapies (e.g., PCSK9 inhibitors, high-dose statins) may significantly reduce LDL/β-Lp levels, resulting in faint or absent bands.
- **Sample Integrity:** Use of non-fasting samples or frozen serum will lead to inaccurate lipoprotein profiles (e.g., presence of chylomicrons or degraded VLDL).
- **State of the Art (Benchmarking):** Per 2025 clinical guidelines (EAS/ESC), visual screening of Lp(a) via agarose electrophoresis remains a valid method for early identification of residual cardiovascular risk where automated systems may lack visual clarity.

11. Troubleshooting

For any deviation from expected results or technical issues, please contact your local distributor or Hellabio directly.

12. Disposal

Dispose of all used and unused reagents, patient samples, and contaminated materials in accordance with local, state, and federal regulations for biohazardous and chemical waste.

13. Classification Statement

This device is classified as **Class B** according to **Annex VIII, Rule 6** of Regulation (EU) 2017/746 (IVDR).
It meets the applicable requirements of **Annex I (General Safety and Performance)** and **Annex II (Technical Documentation).**

14. Manufacturer Information

DIMITRIADIS IOANNIS AND SON PC
Production of electrophoresis products
Business Incubator Thermi
Steliou Kazantzidi 47, 57001, Thermi, Greece
E-mail: hellabio@hellabio.com
Phone: + 30 2311 999911
www.hellabio.com

15. References

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2. **Iammarino, R.M. et al (1969) :** Clin.Chem. 15, 1218-1229.
3. **Klein, G. et al (1970):** Standard Methods of clin. Chem.,6, 127-135.
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6. **Carlson, K. and Carlson L. A. (1975):** Scand. J. clin. Lab. Invest.,35, 655-660.
7. **Herrmann W. et al (1984):** Z. ges. inn. Med. 39,478-481.
8. **EAS Consensus Statement (2024/2025):** "Lipoprotein(a) in clinical practice: Recommendations for screening and risk assessment."
9. **Padelli M., et al. (2025):** "Clinical utility of lipoprotein electrophoresis in the era of new lipid-lowering therapies."

Symbol Panel

Symbol	Meaning
IVD	In Vitro Diagnostic Medical Device
REF	Catalogue Number
LOT	Lot Number
	Use Until/ Expiration Date
	Temperature Limit
	Keep Away from Sunlight
	Manufacturer
	Date Of Manufacture
	Consult Instructions for Use
	Non-Sterile

Symbol	Meaning
	Do Not Reuse
	Unique device identifier
	CE Marking

Agarose Gel Lipoprotein Electrophoresis Kit - Instructions for Use (IFU)

Code	IFU/LE_V16_EN	**Syntax**	Quality Manager
Edition	16	**Approval**	CEO
Manufacturer	Dimitriadis Ioannis and SON PC	**Address**	Steliou Kazantzidi 47, 57001 Thermi, Greece

SYNTAX (NAME-TITLE-SIGNATURE)	APPROVAL (NAME-TITLE-SIGNATURE)
Quality Manager **Gavriilidou Maria**	CEO **Dimitriadis Ioannis**

LIST OF MODIFICATIONS

Revised edition	Date	Description	Adopted by
14	15/01/2026	Addition to Limitations & Interferences	CEO
15	26/03/2026	Updated CE marking statement to include reference to Annex V of IVDR 2017/746 per regulatory requirements.	CEO
16	22/04/2026	Removal of variable fields (LOT/EXP) from the IFU body. Traceability remains ensured via product labeling as per IVDR Annex I, Chapter III.	CEO

The Quality Management Department is responsible for making the process available.

The procedure does not apply if it is not signed by the CEO and the Quality Manager