IVD CE

Instructions for Use (IFU)
Agarose Gel Protein Electrophoresis Kit
REF.: PE

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
PE10	5213012290283
PE15	5213012290290
PE20	5213012290320
PE30	5213012290337
MPE	5213012290245

1. Intended Purpose

The Hellabio Agarose Gel Protein Electrophoresis Kit (PE) is intended for the qualitative separation of human serum proteins based on their electrophoretic mobility in agarose gel. The kit supports clinical evaluation of serum protein patterns, including albumin, α-, β- and γ-globulins.
For **in vitro diagnostic use only**, by qualified laboratory professionals.

2. Summary and Principle of the Method

Protein electrophoresis on agarose gel is a standard laboratory technique used to separate proteins found in biological fluids. When a voltage is applied to an agarose gel saturated with a buffer solution, proteins migrate through the gel. The rate of migration is primarily dependent on the protein's net electrical charge at the given pH of the buffer and, to a lesser extent, its size and shape. After electrophoresis, the proteins are fixed, stained, and visualized as distinct bands. In normal human serum, this results in 5-6 characteristic bands. The Hellabio PE kits utilize a specialized high-resolution agarose and a non-barbital buffer system to ensure optimal separation and reproducibility of results.

3. Reagents and Materials Provided

Component	Content PE10	Content PE15	Content PE20	Content PE30	Content MPE
Agarose Gels	10	10	10	10	12
Electrophoresis Buffer (50X Concentrate)	20 ml	20 ml	20 ml	20 ml	20 ml
Staining Solution (5X Concentrate) Amido Black or Acid Violet	60 ml	60 ml	60 ml	60 ml	20 ml
Protein Diluent Solution (Ready to use)	20 ml	20 ml	20 ml	20 ml	20 ml
Destaining Solution (500X Concentrate)	10 ml	10 ml	10 ml	10 ml	10 ml
Gel Blotter Strips	20	20	20	20	24
Sample Templates	10	10	10	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
- Quality control sera (normal and abnormal levels)
- 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) until the expiration date of the kit.
Staining Solution (Amido Black or Acid Violet)	Dilute the 5X concentrate as instructed on the bottle with distilled water.	Store in a closed flask at room temperature (15–25°C) until the expiration date of the kit.
Protein Diluent Solution	Ready to use.	Store at room temperature (15–25°C) until the expiration date of the kit.
Destaining Solution	Dilute the 500X concentrate as instructed on the bottle with distilled water.	Store at room temperature (15–25°C) until the expiration date of the kit.

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

7. Specimen Collection and Handling

- **Serum:** Use fresh serum. Avoid plasma, as fibrinogen will create a band in the γ-globulin region that can lead to misinterpretation. Collect venous blood into a tube without anticoagulant. Centrifuge to separate the serum. Serum can be stored at 2–8°C for up to 72 hours. For longer storage, freeze at -20°C or below, but note that freezing may alter some electrophoretic patterns.
- **Urine and Cerebrospinal Fluid (CSF):** These samples must be concentrated before analysis to a total protein level of at least 100 mg/dL.

8. Procedure

1. **Sample Preparation:**
   - In case of **Amido black Staining Solution** dilute fresh serum 1:6 with Protein Diluent Solution (e.g. 20 µl serum + 100 µl diluent).
   - In case of **Acid Violet Staining Solution** dilute fresh serum **1:12 with Protein Diluent Solution** (e.g. 10 µl serum + 110 µl diluent).
2. **Chamber Preparation:** Fill the electrophoresis chamber with the required volume of working electrophoresis buffer.
3. **Gel Preparation:** Remove an agarose gel from its packaging. Place it on a clean, horizontal surface.
4. **Blotting:** Blot the sample application zone for 5 seconds using a Gel Blotter Strip.
5. **Template Application:** Place a sample template onto the application zone, ensuring good contact with the gel surface.
6. **Sample Application:**
   - Pipette **5 µl** of the diluted sample into each slit of the template **for PE10/PE15/PE20**
   - Pipette **3 µl** of the diluted sample into each slit of the template **for MPE**.

   Allow 2 minutes for the sample to be fully absorbed.
7. **Excess Removal:** Blot any excess sample with a blotter strip and carefully remove the template.
8. **Electrophoresis:** Place the gel in the tank with the samples on the **cathodic (-) side**. Connect to the power supply and run at a constant:
   - **100 Volts-20 minutes for PE10/PE15/PE20**
   - **100 Volts-18 minutes for MPE**.
9. **Fixing and Staining:** After electrophoresis, completely dry the gel with hot air (<90°C) to fix the proteins. Stain the gel for 5 minutes in the working Staining Solution.
10. **Destaining:** Destain the gel by immersing it in three consecutive baths of working Destaining Solution for 5 minutes each.
11. **Final Drying and Analysis:** Dry the gel again completely with hot air. Clean the back of the film and analyze the results using a densitometer (520-600 nm).

9. Quality Control

It is recommended to run at least one normal and one abnormal control serum with each batch of patient samples. Each laboratory should establish its own acceptance criteria for control materials.

10. Performance Characteristics

The clinical performance of the Agarose Gel Protein Electrophoresis Kit was evaluated using representative normal and pathological serum samples. Electrophoretic patterns were assessed visually by trained laboratory personnel and compared with expected protein distribution profiles.

The method demonstrated reliable separation of albumin, α-, ß- and γ-globulin fractions and enabled the identification of abnormal patterns, including monoclonal bands. Clinical findings showed high concordance with the expected electrophoretic profiles and supported the intended use of the device as a screening tool for serum protein abnormalities.

No false-negative or false-positive electrophoretic patterns were observed in the samples evaluated.

11. Interpretation of Results

- **Qualitative:** Visually inspect the stained protein bands. Compare the patient sample patterns to the normal control for any abnormalities, such as faint or missing bands, extra bands (e.g., monoclonal bands), or changes in the relative intensity of the bands.
- **Quantitative:** Use a densitometer to scan the gel and quantify the percentage of each protein fraction. Compare the results with the established reference ranges.

12. Expected Values

The following reference ranges were established from a population of 100 healthy adults in Northern Greece. It is strongly recommended that each laboratory establishes its own reference ranges.

Serum Protein Fraction	Normal Values (%)
Albumin	52.0 - 65.0
α1-globulins	2.0 - 5.5
α2-globulins	6.0 - 11.7
β1-globulins	4.9 - 9.9
β2-globulins	3.3 - 5.3
γ-globulins	9.5 - 19.8

13. Limitations & Interferences

- Results should always be interpreted in conjunction with the patient's clinical history and other diagnostic findings.
- The use of plasma instead of serum will result in a fibrinogen band in the gamma region, which may be mistaken for a monoclonal protein.
- Aberrant or unexpected patterns should be investigated further using more specific techniques, such as immunofixation electrophoresis (IFE), to identify paraproteins.
- Gross hemolysis may cause a false elevation in the α2- and β-fractions.

Therapeutic Monoclonal Antibodies:

Certain therapeutic monoclonal antibodies (e.g., Daratumumab used in multiple myeloma treatment) are humanized IgG kappa antibodies. These can be detected as a small monoclonal band in the gamma region on serum protein electrophoresis and may be misinterpreted as an endogenous monoclonal gammopathy (false positive M-protein). Interpretation of results for patients under such treatments should be done with caution and correlated with clinical history.

14. Performance Characteristics

- **Reproducibility (Within-Run):** Three different serum samples were run in 10 replicates on the same gel. The Coefficient of Variation (CV%) was calculated for each fraction.
  - **Albumin:** CV < 7%
  - **α1-globulin:** CV < 15%
  - **α2-globulin:** CV < 15%
  - **β-globulin:** CV < 15%
  - **γ-globulin:** CV < 15%
- **Reproducibility (Inter-Run):** Ten different serum samples were run on five different gels from the same lot.
  - **All Fractions:** CV < 5%
- **Accuracy:** A comparison study with another commercially available system yielded a correlation coefficient (r) > 0.93 for all fractions.
- **Linearity:** The assay is linear for total protein concentrations between 0.9 g/dL and 7.2 g/dL.
- **Sensitivity (Limit of Detection):** The lowest detectable concentration was determined to be 80 mg/dL for Albumin and 100 mg/dL for IgG.

15. Troubleshooting

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

16. 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.

17. 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)**.

18. Manufacturer

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19. References

1. **Laurel C.B [1972]:** Scand J clin Lab Invest :29, Suppl, 71-82,

2. **Jeppsson, J.O et al [1979]:** Clin Chem 25 : 629-38.
3. **Killingsworth, L.M [1979]:** CRC Crit Rev in Clin Lab Sci, August
4. **Whicher JT et al [1987]:** Ann Clin Biochem 24: 119 – 32,
5. **Kobayashi S, et al [1995]:** Ann Clin Biochem.;32:63-7.
6. **Kyle RA [1999] Arch Pathol Lab Med 123:** 114 – 8,
7. **Attaelmannan M, et al [2000]:** Clin Chem 46:1230 – 8,
8. **Katzel JA, et al [2007].:** CA Cancer J Clin 57:301 – 18,
9. **Wadhera, Rishi K. et all [2010]:** Review", Mayo Clinic Proceedings. 85 (10): 933-42.
10. **Chhabra S, et al [2013]:** J Lab Physicians.;5(2):145-146.
11. **Rehman, A. A. et al (2013):** J. Cell. Physiol., 228: 1665-1675.
12. **Mcudden C., et al. (2016):** "Interference of Therapeutic Monoclonal Antibody Daratumumab on Serum Protein Electrophoresis and Immunofixation." Clinical Chemistry, 62(2): 347-354.
13. **Keren D.F., et al. (2022):** "Laboratory Implementation of M-Protein Identification and Quantitation." Archives of Pathology & Laboratory Medicine.
14. **Bossuyt X., et al. (2024/2025):** "International recommendations for the detection and characterization of M-proteins." Clinical Chemistry and Laboratory Medicine (CCLM).
15. **Padelli M., et al. (2025):** "Agarose Gel Electrophoresis versus Capillary Zone Electrophoresis: a Comparative Analysis for Monoclonal Protein Detection in Blood." *Clinical Laboratory*.

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
	Do Not Reuse
	Unique device identifier
	CE Marking

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

Code	IFU/PE_V24_EN	**Syntax**	Quality Manager
Edition	24	**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**
22	13/01/2026	Addition to Limitations & Interferences	CEO
23	26/03/2026	Updated CE marking statement to include reference to Annex V of IVDR 2017/746 per regulatory requirements.	CEO
24	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