IVD CE

Instructions for Use (IFU)
Agarose Gel Immunofixation Electrophoresis Kit
REF.: IFE01/IFED01/IFEQ

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
IFE01	5213012290429
IFED01	5213012290269
IFEQ	5213012290436

1. Intended Purpose
The Hellabio Immunofixation Electrophoresis (IFE) Kit is intended for the qualitative identification and characterization of monoclonal paraproteins (M-proteins) in human serum and other biological fluids (e.g., urine). The kit is used to identify heavy chains (IgG, IgA, IgM) and light chains (Kappa and Lambda). It is a diagnostic tool for plasma cell dyscrasias, such as multiple myeloma and Waldenström's macroglobulinemia.
For in vitro diagnostic use only, by qualified laboratory professionals.

2. Summary and Principle of the Method
IFE combines electrophoretic separation with immunoprecipitation. Samples are first separated on an agarose gel according to their charge. Following electrophoresis, monospecific antisera (anti-IgG, IgA, IgM, Kappa, and Lambda) are applied to the individual tracks. If a complementary antigen is present, a stable antigen-antibody complex forms and precipitates within the gel matrix. Unbound proteins are washed away, and the specific immunoprecipitate is visualized by staining.

3. Reagents and Materials Provided

Component	Content IFE01	Content IFED01	Content IFEQ
Agarose Gels	10	10	10
Electrophoresis Buffer (50X Concentrate)	20 ml	20 ml	20 ml
Staining Solution (5X Concentrate)	60 ml	60 ml	60 ml
Protein Diluent Solution (Ready to use)	20 ml	20 ml	20 ml

Component	Content IFE01	Content IFED01	Content IFEQ
Destaining Solution (500X Concentrate)	10 ml	10 ml	10 ml
Gel Blotter Strips	20	20	40
Sample Templates	10	10	20
Antisera Templates	10	10	10
Drying Blotter Sheets	50	50	50
Gel Blotter Sheets	60	60	60
Antisera [Anti IgG(γ), IgA (α), IgM (μ), (f+b) κ- and λ- chain]	Goat anti-Human antisera 1 kit	Goat anti-Human antisera 2 kit	Goat anti-Human antisera 4 kit
Protein fixation solution	1.4 ml	1.4 ml	1.4 ml
Instructions for Use (IFU)	1	1	1

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. Sample Collection and Preparation

- **Serum:** Fresh serum is preferred. Stable for 7 days at 2-8°C.
- **Urine:** Must be concentrated (e.g., 50x to 100x) before application to ensure detection of Bence-Jones proteins.

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	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. Procedure

Extreme care must be taken when choosing the appropriate dilution of the serum sample (100 – 200 mg/dl). The incorrect choice of serum dilution may result in either inability to detect a minor monoclonal protein or a prozone effect. When the protein concentration is low (cerebrospinal fluid, urine), it must be concentrated to get a protein concentration of at least 100 mg/dl.

1. Prepare (with protein diluent solution) freshly dilution of serum sample, so that the concentration of each globulin in corresponded dilution is near 100-200 mg/dl. For example: when the concentration of IgG globulin's is 3000 mg/dl, then the sample should be diluted 1:16 for IgG (187mg/dl). In case of sample with globulin concentration near the normal level, or with unknown concentration, dilute the sample:
    - **for Amido Black Staining Solution** 1:4 (1 vol. serum +3 vol. protein diluent) for PE position and 1:10 for all other positions (1 volume serum +9 volume diluent).
2. Fill the electrophoresis chamber with adequate volume (it depends on the chamber's capacity) 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.
4. Blot the gel for 5'' with a gel blotter strip in the zone of sample application.
5. Place the sample template on the application zone carefully. Rub the template with the forefinger gently to eliminate trapped air bubbles.

6. Using a 5-μl pipette, apply **5 μl of serum dilution** across each corresponding slit [The application of the samples should be done as quickly as possible. The application slits should not be allowed to dry].
7. Let the samples get absorbed into the gel for **2 minutes** and then blot the superfluous sample with a gel blotter strip.
8. Remove both the sample template and gel blotter strip gently and discard them.
9. Place the gel into the electrophoresis chamber with the samples on the cathodic side and run electrophoresis for **20 minutes in 100 Volts** (the time depends on the kind of the power supply).
10. After electrophoresis switch off the power supply and place the gel film in a strictly horizontal position.
11. Blot the gel surface with a gel blotter sheet and apply on the gel surface the antiserum template. Rub the template with the forefinger gently to eliminate air bubbles (very important).
12. Apply into the corresponding troughs of the antisera template:

Position	Reagents	USED VOLUME FOR		
		Kit IFE01	Kit IFED01	Kit IFEQ
PE	Fixation solution	90μl	50μl	50μl
IgG,IgA,IgM, kappa,lambda	Coresponding antisera	50μl	30μl	35μl

13. Incubate the gel film for 10 minutes at room temperature in a moist chamber in strictly horizontal position.
14. Remove the antisera template and discard it.
15. Put on the gel one gel blotter sheet and one drying blotter sheet; place a development weight (about 2 kg) for 5 minutes.
16. Soak the gel in saline solution for 5 minutes.
17. Repeat step (**15**) and (**16**) three more times.
18. Put on the gel one gel blotter sheet and one drying blotter sheet; place a development weight (about 2 kg) for 5 minutes one last time.
19. Clean with a soft paper the back site of the film and dry the gel with hot air (less than 85°C) for 8 minutes and stain it for 5 minutes with protein staining solution.
20. Destain the gel for 5 minutes in three baths of destaining solutions, subsequently.
21. Dry again the gel and evaluate visually the results (see conclusion / troubleshooting).

8. Interpretation of Results

- **Monoclonal Gammopathy:** Defined by a sharp, dense band in a specific heavy chain lane and a corresponding band in a light chain lane (κ or λ).
- **Polyclonal Gammopathy**: Appears as a broad, diffuse staining pattern in one or more lanes.

9. Limitations and Interferences

- **Therapeutic Monoclonal Antibodies:** Patients treated with drugs such as Daratumumab (IgGκ) may show a small, distinct monoclonal band in the IgG and Kappa lanes. This exogenous band can mimic disease recurrence. Clinical correlation is mandatory.
- **Antigen Excess (Prozone):** Very high concentrations of M-protein may result in "hollow" bands. If suspected, repeat the test with a higher sample dilution.

- **Sensitivity:** A negative IFE does not exclude AL amyloidosis. Results should be interpreted alongside **Serum Free Light Chain (sFLC)** assays and/or **Urine Immunofixation (Bence-Jones protein)** is strongly recommended.
- **Method Comparison:** Per 2025 scientific validity data, Agarose IFE remains the **Gold Standard** for visual characterization, offering superior clarity over automated capillary systems for atypical patterns.

10. Performance Evaluation

The Immunofixation Electrophoresis Kit is a qualitative method.
Analytical and clinical performance characteristics have been evaluated during product development in accordance with Regulation (EU) 2017/746 (IVDR).

Detailed performance data are documented in the manufacturer's technical documentation and is available to competent authorities upon request.

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

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15. References

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10. **Keren D.F., et al. (2022):** "Laboratory Implementation of M-Protein Identification and Quantitation." Archives of Pathology & Laboratory Medicine.
11. **Bossuyt X., et al. (2024/2025):** "International recommendations for the detection and characterization of M-proteins." Clinical Chemistry and Laboratory Medicine (CCLM).

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 Immunofixation Electrophoresis Kit - Instructions for Use (IFU)

Code	IFU/IFE_V25_EN	**Syntax**	Quality Manager
Edition	25	**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
23	13/01/2026	Addition to Limitations & Interferences	CEO
24	26/03/2026	Updated CE marking statement to include reference to Annex V of IVDR 2017/746 per regulatory requirements.	CEO
25	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