IVD CE

Instructions for Use (IFU)
Agarose Gel Hemoglobin Electrophoresis Kit
REF.: HE

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
HE10	5213012290344
HE15	5213012290351
MHE	5213012290252

1. Intended Purpose
The Hemoglobin Electrophoresis kit is intended for the separation and identification of normal hemoglobins (HbA, HbA2, HbF) and the detection of major hemoglobin variants (HbS, HbC, HbD, HbE) in human whole blood. The method is used as a screening tool for the diagnosis of hemoglobinopathies and thalassemias.
*For **in vitro diagnostic use only**, by qualified laboratory professionals.*

2. Principle of the Method
Hemoglobins are molecules with different electrical charges. When placed in an alkaline buffer (pH 8.2 - 8.6) and subjected to an electric field, they migrate through the agarose gel at different speeds toward the anode (+). The separation results in distinct bands which are subsequently stained.

3. Reagents and Materials Provided

Component	Content HE10	Content HE15	Content MHE
Agarose Gels	10	10	12
Electrophoresis Buffer (50X concentrate)	20 ml	20 ml	20 ml
Staining Solution (5X concentrate)	60 ml	60 ml	30 ml
Hemolyzing Solution (Ready to use)	20 ml	20 ml	20 ml
Destaining Solution (500X concentrate)	10 ml	10 ml	10 ml
Gel Blotter Strips	20	20	24
Sample Templates	10	10	12
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.
- 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	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.
Hemolyzing 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

Sample: Whole blood collected in EDTA.
Stability: Samples are stable for 3-4 days at 4-8°C.
Preparation: Centrifuge the blood, remove the plasma, and wash the red blood cells (RBCs) three times with saline (0.9% NaCl). Mix 30µl of the packed RBCs with 90µl of the Hemolyzing Solution.

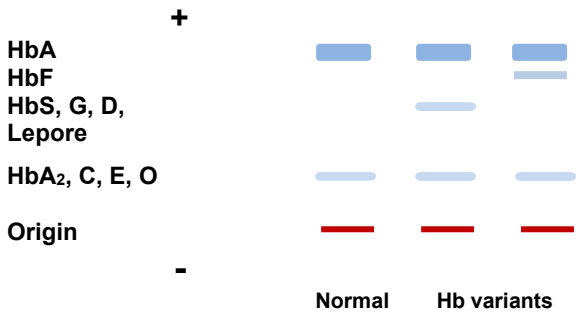
8. Procedure

1. Prepare the hemolysates.
2. Fill the electrophoresis chamber with adequate volume of electrophoresis buffer (it depends on the chamber volume).
3. Take the 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, 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 each hemolysate **for HE10/HE15** across the slits and let absorb for 60 seconds.
   - apply **3 µl** of each hemolysate **for MHE** across the slits and let absorb for 60 seconds.
7. Blot the excess hemolysates 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 with samples on the **cathodic** side; connect the tank to the power supply and run:
   - **30'/200 Volt for HE10/HE15**
   - **20'/200 Volt for MHE**
9. Dry the gel and put it in the staining solution for 5 minutes.
10. Destain the gel for 5 min in three baths of destaining solutions, subsequently.

9. Quality Control

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

10. Exemplary Hemoglobin separation:



11. Interpretation of Results

Normal adult blood shows a major band of HbA (95-98%) and a small band of HbA2 (1.5-3.5%). HbF is typically <1% in adults.

12. Typical Adult Hemoglobin Distribution (Interpretative Guidance)

Hemoglobin Fraction	Normal Values (%)
HbA	96.5-98.5
HbA2	1.5-3.5
HbF	<1

13. Limitations & Interferences

- **Co-migration:** Alkaline electrophoresis is a screening method. Certain rare hemoglobin variants co-migrate at the same position. For instance:
  - **Hb D-Punjab** and **Hb G-Philadelphia** migrate to the same position as **Hb S**.
  - **Hb E** and **Hb O-Arab** migrate to the same position as **Hb C** and **Hb A2**.
- **Confirmation:** In cases where a variant is detected, or if the clinical findings are inconsistent with the electrophoretic pattern, results **must** be confirmed using a complementary method with a different separation principle, such as **Acid Agarose Gel Electrophoresis** or **HPLC**.
- **Sample Quality:** Severely hemolysed or old samples may result in blurred bands.

14. Performance Characteristics

- **Sensitivity/Specificity:** High for the detection of HbA, HbS, HbC, and HbF.
- **Method Comparison:** This agarose gel method shows excellent correlation with Capillary Electrophoresis (CE). While CE provides automation, Agarose Gel Electrophoresis remains the "Gold Standard" for visual confirmation and identification of atypical migration patterns.

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 Information

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19. References

1. **Fucharoen, S et al:** Annals Academy of Medicine. 18, 424-430 (1989).
2. **Wasi, P.:** Clin Haematol 10, 707-29 (1981).
3. **Greene D.N., et al. (2024):** "Laboratory Diagnosis of Hemoglobinopathies and Thalassemia: IFCC Guidelines."
4. **Higgins V., et al. (2023):** "Challenges in the Electrophoretic Separation of Hemoglobin Variants." Journal of Applied Laboratory Medicine.

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 Hemoglobin Electrophoresis Kit - Instructions for Use (IFU)

Code	IFU/HE_V15_EN	**Syntax**	Quality Manager
Edition	15	**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
13	13/01/2026	Addition to Limitations & Interferences and change format to Long Version eIFU	CEO
14	26/03/2026	Updated CE marking statement to include reference to Annex V of IVDR 2017/746 per regulatory requirements.	CEO
15	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 Manage