IVD CE

Instructions for Use (IFU)
Agarose Gel Hemoglobin Electrophoresis in Acid Kit
REF.: GHE

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)	GHE10	MGHE
UDI-DI	5213012290382	5213012290399

1. Intended Purpose
The Hellabio Citrate Agar Gel Kit is intended for the confirmatory separation of hemoglobin variants and the quantification of glycosylated hemoglobin (HbA1). It is specifically used to differentiate HbS from HbG/D/Lepore and HbC from HbE/O/A2 at acid pH (6.0).
*For **in vitro diagnostic use only**, by qualified laboratory professionals.*

2. Principle of the Method
At 6.0 pH, hemoglobins separate based on their interaction with the agaropectin in the agar. This allows for clear differentiation of variants that co-migrate at alkaline pH

3. Reagents and Materials Provided

Component	Content HE10	Content MGHE
Agarose Gels	10	12
Electrophoresis Buffer (33X concentrate)	30 ml	30 ml
Staining Solution (5X concentrate)	60 ml	30 ml
Hemolyzing Solution (Ready to use)	20 ml	20 ml
Destaining Solution (500X concentrate)	10 ml	10 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

- HellabioScan (Gel Analyzer software)
- Precision pipettes
- 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 33X 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

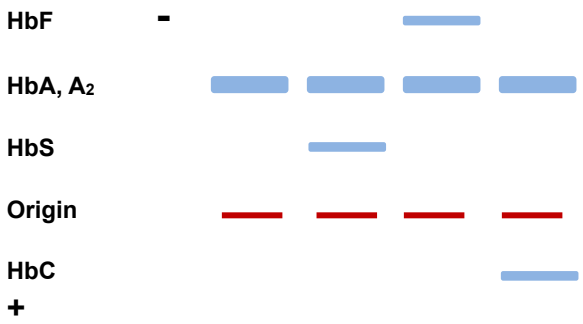
Sample: Whole blood collected in EDTA.
Stability: Samples are stable for 3-4 days at 4-8°C.

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 using a pin from the backside of the plate in the area indicated by the arrow on the top-right, 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:
    - **for GHE10** apply 5 μl of each hemolysate across the slits and let absorb for 60 seconds.
    - **for MGHE** apply 3 μl of each hemolysate 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 on the gel carrier with the gel upstairs and the samples on the anodic side (+); connect the tank to the power supply and run 30΄/ 60 Volts for both GHE10 and MGHE.
9. Dry the gel.
10. **For estimation of HbA1 scan and save the gel without staining. After this step, stain the gel for 2 minutes and correlate the two electropherograms.**
11. For differentiation of hemoglobins stain it for 2 minutes.
12. Decolorize the gel for 5 minutes in three-destaining solution baths subsequently.

9. Exemplary Hemoglobin separation:



11. Interpretation of Results
The qualitative interpretation of the results may be visually interpreted by comparing the sample pattern with the control pattern. For a quantitative interpretation of HbA1 the gel should not be stained and can be measured by densitometer (580nm) or by HellabioScan.

12. Expected Values
HbA_1: 2-8%

13. Limitations & Interferences
- **HbF Monitoring:** Crucial for monitoring patients with Sickle Cell Disease under Hydroxyurea treatment.
- **State of the Art:** Citrate agar remains essential for resolving co-migration issues in automated HPLC/Capillary systems.

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. **Carlson, K. and Carlson L. A. (1975):** Scand. J. clin. Lab. Invest., 35, 655-660.
4. **Noble, R. P. (1968):** Electrophoretic separation of gamma-G and gamma-M hemoglobins. J. of Lipid Research, 9, 693-700.
5. **British Society for Haematology** (2024)

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 in Acid Kit - Instructions for Use (IFU)

Code	IFU/GHE_V18_EN	**Syntax**	Quality Manager
Edition	18	**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
16	19/01/2026	Addition to Limitations & Interferences and change format to Long Version eIFU	CEO
17	26/03/2026	Updated CE marking statement to include reference to Annex V of IVDR 2017/746 per regulatory requirements.	CEO
18	22/04/202+6	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