HEMOGLOBIN ELECTROPHORESIS
on agarose gels (In acid)

IVD CE

Principle and Methodology

The HELLABIO Citrate Agar Gels are suitable for the quantitative determination of total glycosylated hemoglobin [HbA_1], for the separation of HbF and for the differentiation of HbS from HbG, D, Lepore and HbC from HbA2, E,O.

Required Reagents and Equipment's included in each kit:

[Warning: All reagents from each kit must be used together]

Product	KIT GHE10 / 100 TESTS	KIT MGHE / 48 TESTS
Agarose Gels	10	12
Electrophoresis Buffer [33X concentrated]	30 ml	30 ml
Hemolyzing Solution [ready to use]	20 ml	20 ml
Staining Solution [5X concentrated]	60 ml	30 ml
Destaining solution [500X concentrated]	8 ml	8 ml
Gel Blotter Strips	20	24
Sample Templates	10	12
Instructions for use in English		

All reagents must be used according to the instructions and until the expiration date indicated on the kit.

Preparation, storage and stability of the reagents included in the kit:

a) Agarose gels: The Gels are 1.5% agar in non barbital buffer, pH 6.0 and other non- reactive ingredients for long stability and optimum resolution of protein fractions. The Gels must be stored at 15-25 ^{0}C in a horizontal position until the expiration date. Do not freeze the gels. Just before use, carefully remove the gel from the packaging and follow the instructions for use.
b) Electrophoresis Buffer: Non barbital concentrated buffer pH 6.0 and other non-reactive ingredients. It must be stored at 15 – 25 ^{0}C until the expiration date indicated on the kit. **To prepare working solution dilute the content of the bottle with deionized water to a final volume of 1 litre**. The diluted solution is enough for electrophoresis of all gels of the kit. **The buffer solution is for one use only**. Store the diluted solution at room temperature until the expiration date indicated on the kit. If crystals appear, place the vial in warm water to dissolve the crystals.
c) Hemolyzing solution: Ready to use. Store at room temperature until the expiration date indicated on the kit. Store it at 15-25 ^{0}C until the expiration date.
d) Staining Solution: Concentrated Amido Black solution. Store the concentrated solution at 15 - 25 ^{0}C until the expiration date indicated on the kit. **To prepare working solution dilute the content of the bottle with deionized water to a final volume of 300 ml(GHE kits) or 150 ml(MGHE kits)**. The diluted solution is enough for the staining of all gels of the kit.
It should be stored in a closed flask at room temperature until the expiration date indicated on the kit.
e) Gel Blotter strips: Thin filter paper strips to blot the gel in the application area. Blot just for 5 seconds. Avoid humidity.
f) Destaining solution: 2% citric acid solution. **To prepare working solution dilute 2 ml of the content of the bottle with deionized water to a final volume of 1 litre.** Store at room temperature until the expiration date indicated on the kit.

Additional Reagents and Equipments which can be provided by Hellabio:

Controls: Hemolysates of known hemoglobin or commercially available quality control hemolysates should be included in each electrophoresis procedure.
Power supply, Electrophoresis tank, Staining-destaining baths, HellabioScan (Gel Analyzer software).

Limitation / Caution:

- *Do not use the gel if it seems to be dried.*
- *Do not freeze the gels.*
- *Store the agar gels in horizontal position, at 15-25^{0}C*
- *Prefer fresh prepared Hemolysates.*

Preparation of hemolysates:

- *Venous blood from fasting individual is drawn into vacutainer tube with sugar free anticoagulant (oxalated, heparinized or EDTA treated blood).*
- *Add about 100-200 µl of whole blood to a tube with 10 ml saline, centrifuge it and aspirate the supernatant.*
- *Take 15 µl of the sediment and add it to a tube with 90 µl hemolyzing solution and mix it (=Hemolysate). Leave the hemolysate for at least 5 minutes, before proceeding with the electrophoresis.*
- The hemolysate is stable for 3-4 days when stored aseptically in refrigerator avoiding exposure to light.

Interpretation of the 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 HbA_1 the staining should be not stained** and can be measured by densitometer (580nm) or by HellabioScan.

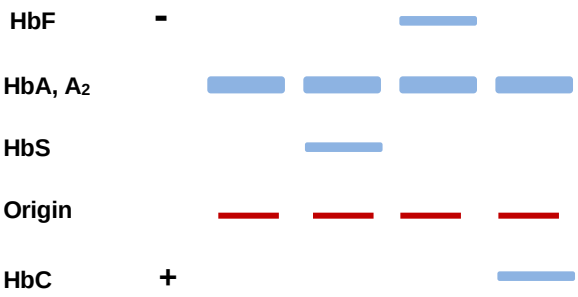
Procedure of Hemoglobin Electrophoresis:

a) Prepare the hemolysates.
b) Fill the electrophoresis chamber with adequate volume of electrophoresis buffer (it depends on the chamber volume).
c) 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.
d) Blot the gel for 5'' with a gel blotter strip, on the sample application zone.
e) Place the sample template on the application zone. Rub the template with forefinger so that it gets contact with the gel surface.
f) Using a 5-µl pipette, apply 5 µl (in case of **MGHE** apply 3 µl) of each hemolysate across the slits and let absorb for 60 seconds.
g) Blot the excess hemolysates with a gel blotter strip, gently remove both the sample template and the gel blotter strip and discard them.
h) 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.
i) Dry the gel.
j) **For estimation of HbA_1 scan and save the gel without staining. After this step, stain the gel for 2 minutes and correlate the two electropherograms.**
k) For differentiation of hemoglobin's stain it for 2 minutes.
l) Decolorize the gel for 5 minutes in three-destaining solution baths subsequently.

Expected values: HbA_1: 2-8%

Precision: In a within-gel electrophoresis of 10-replicated position of a control sample the coefficient of variation values is less than 5%.

Exemplary Hemoglobin (in acid) separation:



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