HEMOGLOBIN ELECTROPHORESIS
on agarose Gels (in alkali)

IVD CE

Principles and Methodology

The HELLABIO Alkaline Agarose Gels are suitable for the identification of different species of hemoglobin molecules (***separation of Hb in distinct band of HbA, HbF, HbS, HbG and HbD, HbA_2 , HbC, HbE and HbO)*** and allow the laboratory diagnosis of hemoglobin abnormalities.

- *Hemoglobin (Hb) is a heterogeneous complex molecule composed of 4 identical polypeptide chains which are linked to a heme (= tetrapyrrolic nucleus linked to an iron atom).*
- *In normal adults the major component comprising about 96-99% of the total Hb, is HbA. A minor fraction accounting for about 1-3,5% is HbA_2. In fetal life the main Hb is HbF, traces (<1%) of which may be found in normal adults too.*
- *Electrophoresis on agarose gel is a suitable technique to separate the various Hb fractions. Each fraction moves in the electrophoresis field according to its charge and molecular size strongly influenced from the pH, ionic strength and the kind of the support.*
- *By this way it is possible to enforce laboratory diagnosis of hemoglobinopathies, such as:*
- ***Hemoglobinopathies** caused by qualitative and or structural changes.*
- ***Thalassemia** cases because of quantitative and or regulation abnormalities.*

Required Reagents and Equipment's included in each kit:
[Warning: All reagents from each kit must be used together]

Product	KIT HE15 / 150 TESTS
Agarose Gels	**10**
Electrophoresis Buffer [50X concentrated]	**20 ml**
Staining Solution [5X concentrated]	**60 ml**
Hemolyzing Solution [ready to use]	**20 ml**
Destaining solution [500X concentrated]	**8 ml**
Gel Blotter Strips	**20**
Sample Templates	**10**
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: Agarose Gels are in non- barbital buffer and other non-reactive ingredients for long stability and optimum resolution of protein fractions. The Gels must be stored at **15 - 25 ^{0}C on horizontal position** until the expiration date indicated on the kit. Do not freeze the gels. Carefully discover the gel just before use and follow the instructions of the manual.
b) Hemolyzing solution: Ready to use. Store at room temperature until the expiration date indicated on the kit.
c) Electrophoresis Buffer: Non-barbital buffer and other non-reactive ingredients. It is in concentrated solution. 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 o the kit. If crystals appear, place the vial in warm water to dissolve the crystals.
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(HE kits) or 150 ml(MHE 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. Avoid humidity. 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 hemoglobins 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 Kit 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 15µl of the sediment and add it to a tube with 90µl 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.*

Calibration:

The estimation of the electropherogram for hemoglobinopathies can be easily done visually.

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 the result densitometer (580nm) may be used.

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, 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 of each hemolysate across the slits and let absorb for 90 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.

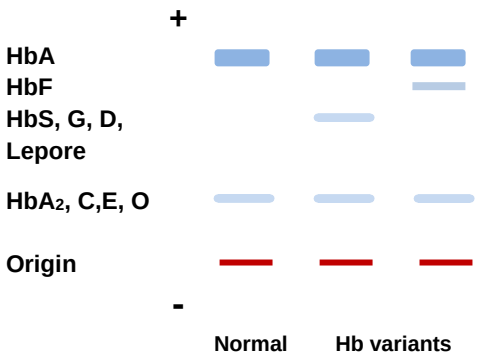
h) Place the gel into the tank with samples on the **cathodic** side; connect the tank to the power supply and run **30' / 200** Volt.

i) Dry the gel and put it in the staining solution for 5 minutes.

j) Decolorize the gel for 5 min in three baths of destaining solutions, subsequently.

*Visit **www.hellabio.com** for video tutorials

Exemplary Hemoglobin separation:



Expected values: HbA: 96.5-98.5, HbA_2: 1.5 - 3.5.

Bibliography:

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