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 HEA13 / 130 TESTS
Agarose Gels	**10**
Sponges	**20**
Buffer Solution [3X concentrated]	**50 ml**
Staining Solution [5X concentrated]	**60 ml**
Hemolyzing Solution [ready to use]	**20 ml**
Destaining solution [500X concentrated]	**20 ml**
Gel Blotter Sheets	**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 for sponges: 3X concentrated stock solution of non barbital buffer and important non-reactive ingredients. To prepare working solution dilute it 1:3 [dilute the buffer to 100ml distilled water]. To use put the sponges on the plastic plate on horizontal position and add to each sponge 6.5 ml of working buffer solution. The working buffer solution can be stored at 4-8 0C until the expiration date indicated on the kit.
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 according to the instructions on the bottle. 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 3 months.
e) Gel Blotter sheets: Thin filter paper strips to blot the gel. Blot just for 5 seconds. Avoid humidity.
f) Destaining solution: 2% citric acid solution. To prepare working solution dilute the content of the bottle according to the instructions on the bottle. 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.

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 30µl of the sediment and add it to a tube with 110µl 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.

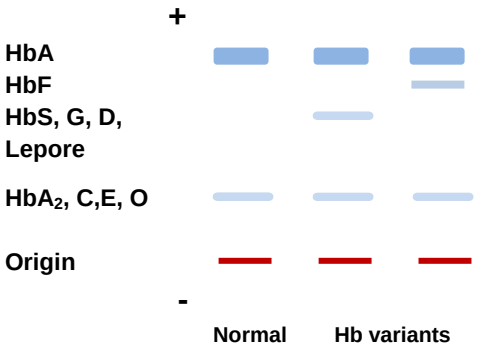
Procedure of automatic Hemoglobin Electrophoresis:

01. Prepare all the reagents (staining-destaining solution) according to the instructions and fill the staining and destaining tank.
02. Fill the distilled water tank with distilled water and 100 ml of tap water.
03. Check that the waste tank is empty.
04. Turn on your computer.
05. Turn on the general switch located on the left backside of the instrument.
06. Then press the power button on the front of the machine (Blue button).
07. Lift the cover of the machine.
08. Take out the sponges from their packaging. Add 6.5 ml buffer on each sponge and apply them on the migration chamber slots.
09. Locate the migration chamber in correct position.
10. Transfer 20-30μl of each sample to the holes of the sample holder.
11. Take the gel out of the plate and put it on the back side of the plate.
12. Blot gently for 5 seconds the surface of the gel with one gel blotter sheet.
13. Apply 0,5ml distilled water on the electrophoresis area.
14. Remove the gel holder and the gel hold support.
15. Fasten the film on the gel holder on the side marked + (anode). Apply the film on the electrophoresis area.
16. Apply carefully the gel hold support on the gel.
17. Double Click on Gemini software.
18. Click on LOGIN.
19. Click on METHODS.
20. Select the type of electrophoresis (Hemoglobin).
21. Adjust the parameters:

Kit			
HEA13			
Phase	Time	^{0}C	mA
Premigration	3’	20	-
Sampling samples	20’’	-	-
Deposition samples	20’’	-	-
Migration	16’	20	220
Desiccation	15’	65	-
Staining	5’	-	-
Destaining (9 cycles)	5’06’’	-	-

22. Save the parameters.
23. Click on the option New Analysis.
24. Select Hemoglobin electrophoresis.
25. Choose the kind of the strip (gel).
26. Choose the number of the samples to be analyzed.
27. Click on the option Confirm.
28. Enter the patient’s data. Click on the option SAVE for each patient.
29. Click on the option START ANALYSIS.
30. Check the status of the machine.
31. Click on the option Continue the process.

Exemplary Hemoglobin separation:



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

Bibliography:

Fucharoen ,S et al : Annals Academy of Medicine. 18, 424-430 (1989).
Wasi, P.: Clin Haematol 10, 707-29 (1981).

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