Quality control

To control the quality of each electrophoretic run you should run one lane with a control serum. The corresponding values to reach are given in the products information leavelet.

Interpretation or results

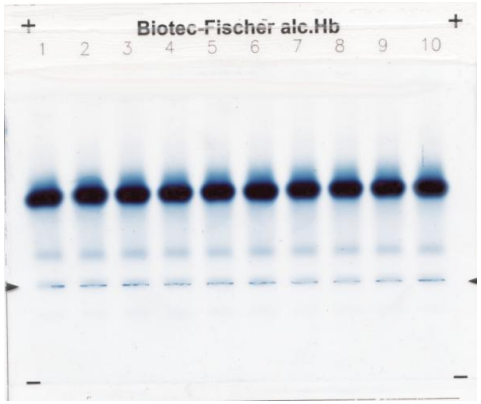
It is recommended that any evaluation of the gels is performed against normal values produced for this method in each individual laboratory.

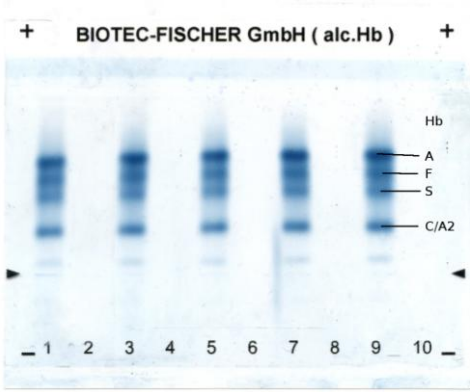
1. **Qualitative evaluation:** The electrophoresis may be inspected for the presence or ablence of particular bands of interest.
2. **Quantitative evaluation:** Run the measurement of the electrophoresis with TurboScan densitometer

In either case, an elevation or decrease in particular serum components or the detection of unusual serum components require further investigation.

Limitation

Since all electrophoresis procedures are non-linear, it is important to follow these instructions for use closely to ensure optimal resolution and reproducible results. Failure to follow these instructions for use may affect the results obtained.





PHERO-trol AFSC

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Manual

Alc.Hb Electrophoresis on Biotec-Fischer Agarosegel

for product code
373601-001 / 373601-002
373601-101 / 373601-102

Intend purpose

The folllowing named reagents are only used to migrate alcalic hemoglobins on agarose gels

The hemoglobin kit is intended for the electrophoretic Separation of human hemoglobins to screen for clinically important hemoglobine variants. Alcaline hemoglobin electrophoresis on agarose gel is used as a screening procedure for hemoglobins A, F, S and C. The principle of electrophoresis is based upon the fact that hemoglobins, when placed in an electrical field, will migrate toward one ofthe electrode poles.

Warning

All reagents are for in vitro diagnostic use only. Don not ingest or pipette by mouth any component. Refer to the product safety sheet for component risk and safety phrases in disposal information.

Necessary Equipment

Reagents

Prod. No.:	Name
373601	**Kit for 100 alcHb Electrophoresis on Agarosegel** Content: 1 x 10 agarose gels for 10 tests per gel 1 x 10 thin sheet drying paper for agarose gels 1 x 10 sample templates 1 x 10 drying paper for sample templates 1 x 100 ml alcHb buffer concentrate *Dilute buffer with 900 ml with Aqua dest.* 1 x 50 ml Amido Black staining concentrate *Dilute one bottle with 200 ml Aqua dest.*

Equipment

Prod. no.:	Name
321001	Electrophoresis tank model 1000
321000	*Electrophoresis tank model BFA (altern.)*
321101	*Electrophoresis tank FILIPO*
341225	Staining vessel kit for agarose gels
332010	Drying oven KB19
313001	MAESTRO 101 (no further equipment necessary)
331000	Densitometer TurboScan

Reagents which are not part of kit

Name
Fixativ
Ethanol : dist. Water : Acetic acid (50 : 40 : 10)
Destaining
3% Acetic acid

Shelf live

Unopened agarose gels will be usable until shelf live marking on the envelope

Sample preparation

Freshly collected serum is the specimen of choice.

Sample preparation:
A red blood cell hemolysate is the best choice for the routine investigation of hemoglobin. The blood should be collected in EDTA, citric acid or heparin anticoagulants. When stored at +2°C to +8°C whole blood is stable for 7 days. Centrifuge sample for 5 minutes to separate cells from plasma

1. Remove plasma and buffy coat by aspiration
2. wash packed red cells with saline solution. Dilute 200 µl cells with 1000µl saline (0.85% NaCl). Centrifuge at 3000 rpm for 5 minutes
3. Remove buffy coat by aspiration
4. Repeat #2-3 for 2 more times

Dilute the washed cells between 1/10 to 1/30 with double-distilled water. The final concentration should be 2-2.5 mg/dl total hemoglobin.

Step by step procedure

1. Fill the first vessels with fixation solution, the second with staining solution, the next 2 vessels with destaining solution.
2. Fill one half of migration tank model 1000 with buffer solution until it flows over into the other half. (Fill 35 ml buffer solution in each cavity of Filipo or MAESTRO 101 migration unit)
3. Lift one side of the tank 1000 so both sides (cathode and anode) will reach the same filling level.
4. Dry the middle separator with a paper wick.
5. Take carefully a gel out of its package and place it flat on the table with gelside up and carefully remove protection foil without damaging the gel

Step by step procedure (continued)

6. Take one Gel blotting paper wick and place it carefully on the agarose gel to remove the liquid on gels surface for 1 seconds. Afterwards remove the paper wick carefully without damaging of lifting off the gel from the support.
7. Place the application template on the marking at cathode (-) side
8. Position the small paper wick on the application slids and press gently to remove air bubbles between template and gel.
9. Fill each slid of the serum plate with 3 µl hemoglobin (one spot in the center of each slid!)
10. Wait for 3 minutes and let the samples enter the gel.
11. Position the small paper wick on the application slids to remove the left sample from the template and lift template together with small paper wick from the gel.
12. Place the gel into gelbridge with gelside down. Use inner positioning guide.
13. Place the gel bridge into the tank. If necessary fill up cathode and anode side of tank with buffer solution until the gel has direct contact with buffer solution
14. Close the lid of migration tank. Take care of correct connection of cathode and anode. (Application side has to be connected to cathode (-) black colored)
15. Run the electrophoresis at 150 Volt for 30 minutes
16. After migration remove the gel from the gel bridge and insert it into the frame of staining vessels and place it for 5 minute in the first bath (fixation solution).
17. Remove the gel from fixation solution, remove it from the vessel frame and dry it in the drying oven at ~56°C hot air.
18. Place the gel back into the frame of staining vessels and place it for 5 minutes in the second bath (staining solution).
19. After staining place the gel into bath 3 and 4 (destaining solution each) for 5 minutes each.
20. After destaining dry the gel again for approx. 10 minutes at ~56°C hot air
21. Calculate the gel with TurboScan densitometer