

Haemoglobin Electrophoresis Kit 200 Tests (Cat # GRC-955)

Electrophoresis is a commonly used technique to separate haemoglobin fractions. It can provide useful diagnostic information about most types of thalassaemias and abnormal haemoglobins. This kit is designed to provide good quality results of haemoglobin electrophoresis on the GTI-Haemoglobin Electrophoresis Apparatus.

Kit Contents

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| 1. Cellulose Acetate Electrophoresis Strips (37 X 57 mm) | X 4 |
| 2. Electrophoresis Buffer (1 X) | For 1L |
| 3. Elution Buffer (1 X) | For 1L |
| 4. Lyse Reagent | 25 ml |
| 5. Carbon Tetra Chloride (CCl ₄) | 20 ml |
| 6. Filter Paper Wicks | X 4 |

Material required but not provided in the kit

1. Eppendorf Tubes (1.5 ml)
2. Eppendorf Centrifuge or Bench Top Centrifuge with tube adaptors.
3. Disposable plastic syringe (20 ml).
4. Adjustable Pipettes (1-10 µl and 20-100 µl)
5. Yellow Tips
6. Two plastic containers with lids (500 ml capacity each)
7. Forceps

Preparation of Haemolysate

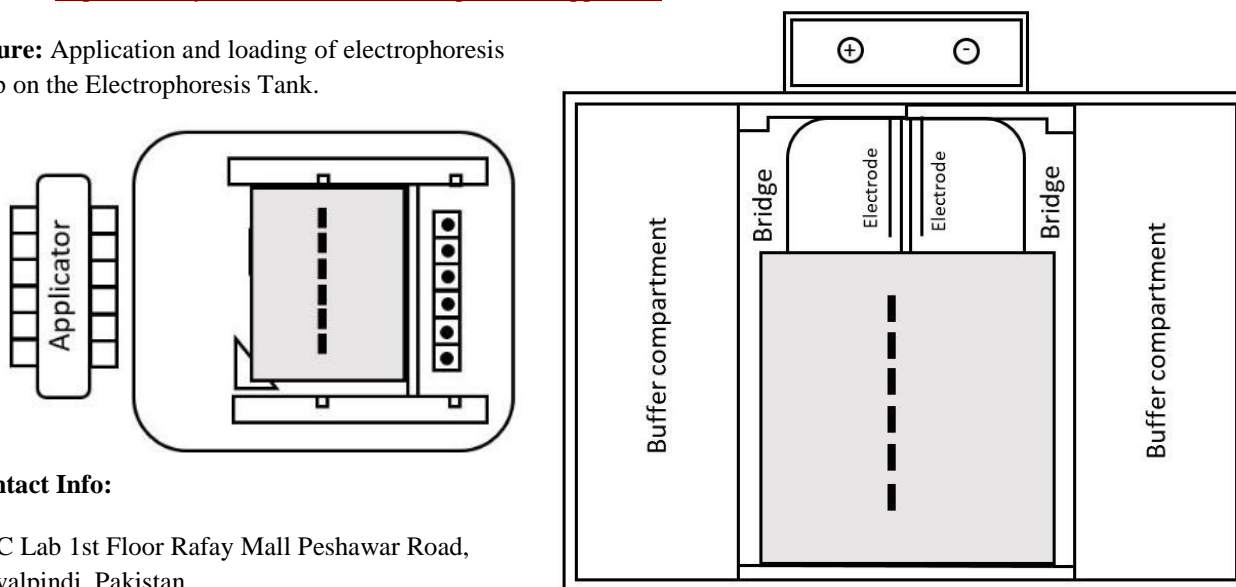
1. Take 2-3 ml venous blood in EDTA tube. For best results use fresh blood sample.
2. Centrifuge the blood sample at 3000 rpm for 1-2 minute.
3. Take 75 µl of packed red cells in labelled Eppendorf tubes taking care to avoid drawing any plasma. Since the packed red cells are thick in consistency the pipette tip should be dipped in the red cells for at least 5-10 seconds to ensure complete drawing of red cells. Similarly the red cells should be pipetted out in at least five seconds.
4. Add 75 µl of Lyse Reagent to the packed red cells and mix by tapping the tubes.
5. The ratio of packed red cells to lyse reagent may vary from 1:1 to 1:2.
6. Add 50 µl of Carbon Tetra Chloride (CCl₄). CCl₄ is highly volatile therefore its bottle should be securely capped to avoid any evaporation.
7. Close the Eppendorf tube lids tightly and shake well by vortex mixer or vigorous tapping.
8. Centrifuge the Eppendorf tubes at 3000 rpm for one minute.
9. For electrophoresis use the clear supernatant haemolysate.
10. The haemolysate is stable for 6-12 hours at 4°C. For longer storage it is best to store the whole blood at 4°C and prepare the haemolysate just before use.

Electrophoresis

1. Reconstitute the electrophoresis buffer in 1L of distilled water.
2. Take about 200 ml buffer in a plastic container with lid.
3. Dip the Cellulose Acetate Electrophoresis strip(s) in the buffer initially for at least 12 hours. Thereafter the strips may be kept permanently dipped in buffer.
4. Apply the filter paper wicks on each bridge of the electrophoresis tank.
5. Fill the electrophoresis tank with 20 ml buffer by a disposable syringe making sure that the filter paper wicks are well soaked with the buffer. The amount of buffer in the tank is critical. Over or under filling of the electrophoresis tank may give erroneous results. In between the runs the buffer may evaporate. This can be avoided by withdrawing the buffer in the 20 ml disposable syringe and kept for reuse. Fresh buffer is good for 5-6 electrophoresis runs after which it should be discarded or mixed with the elution buffer.
6. Equalize the level of buffer in the two compartments by gentle forward tilting of the electrophoresis tank.

7. Place the sample applicator on a horizontal surface and carefully take 5 μ l from the top most portion of each haemolysate. Pour the haemolysate(s) on the sample tile(s) taking care to avoid any bubble formation.
8. It is best to process the samples in a batch of 4-6 including a freshly prepared control from a thalassaemia trait (minor) blood sample.
9. Gently place the clean dried sample comb on the sample tile and allow even spreading of the haemolysate under the teeth of the comb by gently rocking the comb to and fro or sideways.
10. Carefully take the soaked electrophoresis strip out of the buffer with forceps and wipe the excess buffer by pressing between the two layers of a filter paper.
11. Place the electrophoresis strip in the designated space on the sample applicator making sure that it aligns with the margins of the designated space on the applicator (Figure).
12. Pick the loaded sample comb and carefully place it in the slot over the electrophoresis strip. Let the comb drop on the electrophoresis strip without applying any extra pressure. With a little bit of practice evenly spread sample application can be ensured.
13. Pick the loaded electrophoresis strip with forceps and place it across the two bridges of the electrophoresis tank as shown in the figure.
14. The loaded electrophoresis strip may get partially dried during its transfer from the applicator to the electrophoresis tank. It is perfectly normal and the strip's moisture is regained immediately after its placement in the electrophoresis tank.
15. Replace the lid of the electrophoresis tank and turn the power supply on. The start of electrophoresis is indicated by illumination of green LED in the electrophoresis tank.
16. The sample applicator tiles and the comb must be thoroughly washed with running tap water as the haemolysate on these may get dried. The applicator and the comb should be gently dried with tissue paper.
17. The power supply is equipped with a built in timer of approximately 15 minutes. The power supply can be restarted if a run longer than 15 minutes is required.
18. Since Haemoglobin is a coloured compound its analysis can be done without any staining. At the end of electrophoresis the results can be saved by taking a picture with the GTI-Image Capturing Device.
19. Fraction analysis of the electrophoresis runs can be done with the GTI-Thal-IT® App.
20. The unstained electrophoresis strips can be reused for at least five to ten times without losing quality. The used strips are dipped for at least 30 minutes in about 200 ml of elution buffer (prepare by dissolving the contents of the packet in the kit in one liter of water) in a plastic container. Once the haemolysate is washed away the strip surface may be cleaned by gently rubbing with finger. The washed strips must be kept in the electrophoresis buffer for at least 30 minutes before reusing.
21. A video on how to use the Haemoglobin Electrophoresis Kit and the apparatus may be accessed at: [https://www.youtube.com/Hb Electrophoresis Apparatus](https://www.youtube.com/Hb%20Electrophoresis%20Apparatus)

Figure: Application and loading of electrophoresis strip on the Electrophoresis Tank.



Contact Info:

GRC Lab 1st Floor Rafay Mall Peshawar Road,
Rawalpindi, Pakistan

Phone: +92-51-5167312, 051-8350835; E-mail: info@thal-it.com; Website: www.thal-it.com