

**SAIO**  
**Hemoglobins Kit 200 Tests**  
**Electrophoresis Procedure**

**REF HBA22S**

**Intended use**

The Alkaline Hemoglobin Electrophoresis (Hb) kit is intended for the qualitative and semi-quantitative determination of both normal Hemoglobins and abnormal or variant Hemoglobins by electrophoresis on cellulose acetate strips. The electrophoretic test is performed at alkaline pH and provides a valuable screening method for Hemoglobin patterns. The kit is used with the automated Saio instruments.

**Summary**

Hemoglobin is a protein that is found in red blood cells and plays a major role as a carrier for oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>).

Hemoglobin picks up oxygen in the lung to form oxyhemoglobin, that releases oxygen in tissues and organs and binds carbon dioxide. The carbon dioxide bound form of hemoglobin is called carbaminohemoglobin and transports CO<sub>2</sub> to the lungs. In the red blood cells of a normal adult three different types of hemoglobin exist. HbA is the major type with small amounts of HbA<sub>2</sub> and HbF.

Electrophoresis on cellulose acetate at alkaline pH is a simple and useful method for the separation, the qualitative identification and semi-quantitative determination of different types of hemoglobins. Up to 400 variant hemoglobins have been identified in human. About one fourth of these abnormal hemoglobins can be identified by classical analytical methods like electrophoresis performed with alkaline or acid buffer solution; investigation of the other types of hemoglobin often requires highly sophisticated laboratory techniques.

Hemoglobinopathies and thalassemias comprise a large number of hereditary disorders that can produce either qualitative modification of hemoglobin structure, or quantitative variations of hemoglobin synthesis that eventually lead to an imbalance of the normal concentration of the different types of hemoglobins. The two mutant hemoglobins most commonly seen are HbS and HbC. Hb Lepore, HbE, HbG-Philadelphia, HbD-Los Angeles, and HbO-Arab may be seen less frequently.

**Principle**

Normal and 'variant' hemoglobins display different electrophoretic mobility and can be therefore identified by zone electrophoresis performed on cellulose acetate. The electrophoretic pattern of a normal adult displays only physiological hemoglobin types that include HbA, HbF, and HbA<sub>2</sub>, whose concentration falls within normal ranges (see Reference Range Table).

Hemoglobin anomalies of major clinical interest are evident from the qualitative identification and quantitative determination of different types of hemoglobins. The detection of abnormal bands indicates the presence of variant hemoglobin in the blood sample.

**Warning: This kit is for in vitro diagnostic use only.**

**Specimen Collection and Handling**

Whole blood samples should be collected using the laboratory's procedures and in accordance with Good Laboratory Practice (GLP) Guidelines. Whole blood collected with EDTA is the specimen of choice. Whole blood and packed red cells may be stored up to one week if stored at 4...6°C. The hemolysate should be prepared fresh on the same day the electrophoresis is performed. Hemolysates are stable at 4...6°C for 8 hours after preparation.

**Reagents**

Reagents are supplied in concentrated solutions and ready to

use. Please reference the operator's manual for the correct addition of reagents to the Instrument reagent rack.

**Storage and stability:** Store all reagents at room temperature (15° to 30°C). All reagents are stable until the expiration date indicated on the label.

**Strips**

Cellulose acetate supported on Mylar<sup>®</sup>

**Buffer (Ready to use solution)**

Contains: <40% Tris Base

**Staining Solution ready to use**

Contains: Ponceau S Red, <5% Acetic acid

**Destaining (concentrated solution)**

Contains: Citric Acid <50%

**Warning:** Irritant for eyes and skin

**Items Provided**

Kit product number: **HBA22S** For **SAIO** instrument 200 tests.

Ref	Description	Q.ty
AC4C25	Strips - Dry Plates SET	2
BRU52HB	Buffer ready to use 500 mL	1
SRP2AC5	Staining Solution 250 mL	1
DES2AC5	Destaining Solution 250 mL	2
BPS13	Blotting Paper Set for Saio	1

**Reagents and Materials Required But Not Provided**

- Serological pipettes. Preferably pipetting devices 10-100µL
- Saline solution 0.9% NaCl
- Centrifuge
- Vortex
- Distilled Water.
- Hemoglobin Control

**Test Procedure**

**Sample Preparation**

*Washing of red blood cells (RBCs)*

1. Centrifuge the total whole blood at 3000 rpm for 5 minutes.
2. Dispense 1 ml of saline - 0.9% (w/v) NaCl - in a 1.5 ml conical test-tube.
3. Dispense 100 µl of the red blood cells at the bottom of the tube.
4. Centrifuge at 3000 rpm for 5 minutes
5. Remove supernatant
6. Add 1 ml of fresh saline, re-suspend RBCs gently and repeat centrifugation step at 3000 rpm for 5 minutes.
7. The washing is considered complete when supernatant is clear and colorless. If supernatant still appears pale red to red after three cycles of washing, eliminate the sample.

*Lysing of RBCs*

1. Remove supernatant making sure that no residual saline remains on the "pellet" of RBCs
2. To one volume of washed RBCs add five volumes of distilled water. For example, add 250 µl of distilled water to 50 µl of washed RBCs. **IMPORTANT:** final concentration of hemoglobin should be within the range of 2.5 to 3.0 g/dl.
3. "Vortex" for about 30 seconds.
4. Centrifuge at 5000 rpm for 5 minutes. This solution that contains hemoglobin is the **hemolysate** used for the electrophoresis.

*Pipetting Hemolysate Sample*

The hemolysate sample may take on two different appearances:

- a) The liquid in the test tube has a clear, red solution in the



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upper part of the liquid column with a dense, opaque, gel-like solution in the lower part of the test tube. The clear, red solution is the part of the sample to be used and sampled for the analysis. **Precaution:** Do not use the dense opaque, gel-like liquid. Avoid mixing the two parts of the sample while pipetting from the test tube.

b) The liquid in the test tube appears uniformly red and clear. Pipette only from the **first half or top** of the clear red liquid sample in the test tube.

#### Migration Chamber and Reagents

Insert Blotters in the slots of the migration chamber. Complete the preparation of Reagents as indicated on the Operator's Manual. Load diluted Buffer, Ponceau ready to use solution, diluted Destaining solution and Distilled Water in each external tank. Check the level of waste tank, it must be empty.

#### Strips Positioning

Place the strip on the strip holder as shown in the Operator's Manual. Do not touch the acetate side with fingers.

#### Samples

Dispense 35 µL of sample into each well of the sample plate. Avoid introducing air bubbles. Bubbles and foam can interfere with results.

#### Strip Holder

Place the blotter into the correct position as shown in the Operator's Manual. Load the sample plate onto the instrument.

#### Electrophoresis Conditions

Check on the instrument that the HEMOGLOBIN migration conditions are correct for the specific test. Consult the Operator's Manual for further information on setting.

#### Start the run

Consult the operator's manual for further information about the starting procedure.

#### Additional note

Blotting Paper for migration chamber must be changed after 25 electrophoretic runs.

Blotting Paper for strip drying must be discarded after 5 runs.

Blotting Paper for sample plate must be discarded after 3 runs.

#### Test Results

- HbA is the predominant type of hemoglobin in the red cell, therefore is the prevalent band in the pattern. HbA can be easily recognized. It has a regular shape with uniform profile on its cathode side and a mild smearing on its anodal side.

- HbA2 band is typically small and focused, although mildly stained.

- HbA and HbA2 bands are well resolved and distant, with colorless background between them.

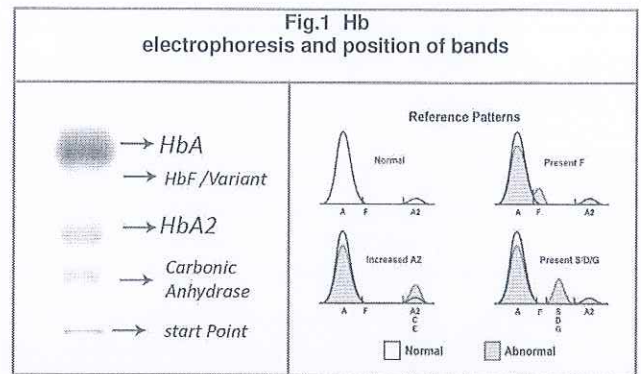
- The carbonic anhydrase band is small and well focused. Intensity of staining is constant even in those clinical conditions displaying dramatic variations of the concentration of the hemoglobin fractions.

- HbA2 and carbonic anhydrase bands are well resolved and distant. Focusing and resolution of these two bands are important markers of the quality of the electrophoretic pattern of the hemoglobins.

- The point of sample application should NOT be visible in the pattern. If the application point is present as a line mildly to strongly stained, expect a smeared pattern, with poor resolution and substandard focusing of the bands.

- Identity of the resolved bands is assigned according to their position in the pattern. The determination is made by a comparison of the pattern of the patient sample with that of a reference hemoglobin control. The reference hemoglobin control typically should contain a mixture of HbA, HbF, HbS and HbA2.

- Graphs obtained from densitometry of hemoglobin patterns for quantitative evaluation should never include carbonic anhydrase.



#### Reference Values

In the following table, the Hb reference ranges were established with Hb kit on **Saio** instruments. These values are presented as a guideline. Each laboratory should establish its own reference values.

Fraction	Reference range (%)
HbA	96 - 98
HbF	≤2
HbA2	1.5 - 3.5

#### Quality control

It is recommended to include controls with the patient sample runs in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. The laboratory should establish acceptance parameters for each lot of control material. If the controls are not within these parameters, patient test results are suspect and the analysis should be repeated.

#### Bibliography

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**The compliance of the kit with the directive 98/79/CE and related performance described in the procedure, are guaranteed only if the products are used according to our instructions of use**

SIMBOLI E SIGNIFICATO		
REF PRODUCT CODE	LOT LOT NUMBER	READ THE INSTRUCTIONS
EXPIRATION DATE	STORAGE TEMPERATURE	MANUFACTURER
DO NOT RE-USE	IVD IN VITRO DIAGNOSTIC	CE MARK
CONF CONFECTION		