



HEMOGLOBIN A₂

Chromatographic determination of Hemoglobin A₂
(HbA₂) in blood



ORDER INFORMATION

REF	Kit size
GD1050 00	25 determinations

INDICATION

High levels of HbA₂ are typical symptoms of a genetic anomaly known as β-Thalassemia. Such disease results from a decreased synthesis of β-chains which at the homozygous state (β-Thalassemia Major) causes severe anemias, while at the heterozygous state (β-Thalassemia Minor) causes a mild impairment. A smaller quantity of hemoglobin β-chains is synthesized in this conditions and then the HbA₂ level, containing δ-chains instead of β-chains, is higher. For this reason the HbA₂ determination allows an accurate diagnosis of anemia caused by such anomaly and points out possible genetic risks.

PRINCIPLE

The hemolysate is directly placed in the test tubes containing DEAE-cellulose resin. Hemoglobin A₂ unlike all remaining hemoglobin is not linked by the resin and then can be separated by means of special separating filters.

COMPOSITION

REAGENT A:

Resin	25x2.5 ml
DEAE-cellulose, preweighted in tube	

REAGENT B:

Lysing Solution	1x20 ml
Triton X100	

FILTERS SEPARATORS:	n. 25
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Reagents preparation

The reagents are ready to use.

Storage and stability

The reagents are stable up to the expiry date stated on the labels, if stored at 15-25 °C. Strong temperature variations may alter resin equilibrium and consequently its functionality; if erroneously stored at 2-8 °C the resin has to remain at room temperature for at least three days before using. Tubes containing yellowish-white resin indicate chemical degradation and cannot be used.

ANCILLARY EQUIPMENT

- Semi automatic pipettes of 20-1000 µl
- Rocker or hematology rotator
- Chronometer
- Glass or plastic test tubes to hold 0.3 ml and 10 ml.
- Spectrophotometer or colorimeter set at 415 nm.

SPECIMEN

Whole blood with heparin or EDTA.
Stability: Hb A₂ is stable 7 days in blood stored at 2-8 °C or 15 days in the hemolysate frozen at -20 °C.

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

The reliability of test results should be monitored routinely using stable quality control materials and analyzed in the same manner employed for the unknowns.

PROCEDURE

Allow reagents to reach working temperature before using.

Hemolysate Preparation:

1. Dispense into tube 300 µl Lysing Solution (Reagent B).
2. Place 50 µl of the well-mixed blood sample.
3. Mix well and allow to stand for 5 minutes.

Haemoglobin A₂ preparation:

1. Add 100 µl of the hemolysate in the resin tube (RA).
2. Position the Filter Separators in the tubes so that the rubber sleeve is approximately 1 cm above the liquid level.
3. Place the tubes on the rocker or rotator and gently mix continuously for 5 minutes (alternatively turn upside down at least six times at intervals of one minute).
4. Remove the tubes from the rocker or rotator. Push the Filter Separator into the tubes proceeding slowly until the resin is firmly packed.

Step 1



Step 2



Step 4



5. The supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.
6. Read the absorbance values at 415 nm against a reagent blank made of liquid phase obtained from a test tube without hemolysate (A HbA₂).

Note:

- It is recommended to let the resin stand for one minute after mixing.
- During the separation the filter has to be introduced slowly to avoid a strong pressure let micro particles, always present in the mixture, pass through the supernatant with consequent increase in turbidity and HbA₂ overestimation.

Total Hemoglobin Fraction:

1. Dispense 20 µl ml deionized water into tubes containing 10 ml of distilled water. Mix well.
2. Read absorbance values at 415 nm against distilled water (A Hb Tot).

CALCULATION OF RESULTS

Results should be determined as follows:

$$\% \text{HbA}_2 = \frac{A \text{HbA}_2}{A \text{Hb Tot} \times 22} \times 100$$

EXPECTED VALUES⁽¹⁾

	Values (%)
Control subjects	1.8 ÷ 3.2
Heterozygous β-Thalassemia	3.2 ÷ 5
Homozygous β-Thalassemia	>5

Each laboratory should establish reference ranges for its own patient population.

ANALYTICAL PERFORMANCES

Within run precision

n=30	mean (%)	SD (%)	%CV
Sample 1	2.14	0.174	8.1
Sample 2	4.17	0.175	4.2

Between run precision

n=30	mean (%)	SD (%)	%CV
Sample 1	2.23	0.185	8.3
Sample 2	4.25	0.170	4.0

Linearity

Linearity has been tested on serial dilutions of a blood sample with high concentration of HbA₂ (5%) with a sample with a low concentration (2.5%). The results obtained are the following:

$$y = 1.089x + 0.267 \quad r = 0.997$$

Correlation

A comparative study of the present method and a commercial one gave the following results:

$$y=0.9791x+0.0202\% \quad r=0.996$$

Interferences

Pathological hemoglobins (S, C, E, O, D, G) may interfere in the determination.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended⁸.

Waste management

Please refer to local legal requirements.

REFERENCES

1. HENRY J.B., Clin. Diagnosis and Management. 17th edition, Saunders Publisher (1984).
2. ABRAHAM E.C., REESE A., STALINGS M. and HUISMAN T.H.J. Hemoglobin 1:27 (1976-1977).
3. HUISMAN T.H.J., SCHROEDER W.A., BRODIE A.N., MAYSON S.M. and JAKWAY J. Lab. Clin. Med. 86: 700 (1975).
4. MARINGONI A., TORELLI G. Quad. Sclavo Diagn. 21:135 (1985).
5. MARINGONI A., TORELLI G.; 17th Meeting SiBioc; Bioch. Clin. 9:1055 (1985).
6. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
7. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

