EU rev. G11B-0102001

Tosoh Automated Glycohemoglobin Analyzer HLC-723®G11

Instructions For Use

G11® β-Thalassemia Elution Buffer No.1 (S)
No.2 (S)
No.3 (S)

CE

TOSOH CORPORATION

TOSOH

Attention: **This IFU complies with IVD directive 98/79/EC and is intended for use by customers operating in a member state of the European Union**

Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this IFU thoroughly before using the product.

[Notational Convention]

Notation	Explanation
⚠ **CAUTION**	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

⚠ CAUTION

- **Use only in well ventilated areas**

  In case of insufficient ventilation, spilled solvents can cause harm.

- **First Aid**

  Skin exposure

  Wash exposed area with plenty of soap and water.

  Eye exposure

  Open eyes as wide as possible and wash with clean water for at least 15 minutes.
  Immediately call for medical attention.

  Ingestion

  Please wash mouth with excess water and immediately call for medical attention.

- **Do not spill solvents**

  Spillage and leakage can cause fire, electric shock, poisoning, injury, and corrosion.
  Wear appropriate protective gear when cleaning up a spill.

- **Wear eye protection and protective gloves**

  Organic acids are harmful and should not come in direct contact with the skin.

- **Handle package with care**

  Inappropriate handling may cause rupturing and/or spattering of the product.

- **Only use this product as intended**

  This product is intended for *IN VITRO* DIAGNOSTIC USE ONLY for the measurement of Hemoglobin F and A_2 in blood specimens by healthcare professionals.

- **Proper disposal**

  Dispose in accordance with local, state and federal laws and regulations.

NOTE

Keep this IFU with the product for future reference

CONTENTS

Published in 03/2017

1. Intended Use

The G11 β-Thalassemia Elution Buffers are intended for *IN VITRO* DIAGNOSTIC USE for the quantitative percent determination of Hemoglobin F and A_2 and for the detection of the major haemoglobin variants, especially HbE, HbD, HbS and HbC, in whole blood samples using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G11, β-Thalassemia Analysis Mode (referred to as HLC-723G11 in this IFU), which is based on the principle of high-performance liquid chromatography assay, to aid in the diagnosis of β-thalassemia. It is not designed for and should never be used with any other type of system. The G11 β-Thalassemia Elution Buffers are intended for Healthcare Professionals Use Only.

2. Summary and Explanation of the Test

Hemoglobin(Hb) is a tetramer which is composed of 2 α-chains and 2 non α-chains. In the normal synthesis, α-chains and non α-chains (β-chain, γ-chain and δ-chain) are synthesized with no overproduction or underproduction.
Thalassemias are hereditary disorders that are caused by abnormally synthesized globin chains.
Since Thalassemia carriers are frequently found in a broad belt around the Mediterranean, it's sometimes called "Mediterranean anemia".
In Thalassemic disorders, due to a quantitative defect in synthesis of a specific chain, free α chains or single globin chains such as HbH (β4), HbBart's (γ4) of hemoglobin could be synthesized.
β-Thalassemia in an analogous manner means reduced or missing production of hemoglobin β-chains, which is compensated by overproduction of γ-chains and δ-chains. Consequently, HbA_2 (α2δ2) and HbF (α2γ2) are often elevated in β-Thalassemia.

3. Principle of the Procedure

The HLC-723G11 is based on the High-Performance Liquid Chromatography (HPLC) principle. Separation is obtained with a cation exchange column based on differences in ionic interactions between haemoglobin components within 5.0 min.
A step gradient elution is used to separate and assay HbF and HbA_2. The three-types of G11 β-Thalassemia Elution Buffers (G11 β-Thalassemia Elution Buffer No.1, 2, and 3 (S)) contain different salt concentrations and pH. HbF (%) and HbA_2 (%) are reported as a relative percentage of the integrated area of each Hb fraction against the sum of those haemoglobin fractions, after being calibrated using the calibration curve established with G11 F&A2 Calibrator Set.

4. Before Use

Inspect the packaging and the exterior of the aluminium pack for any signs of damage before use. If any damages are visible, contact your local Tosoh sales representative.

Confirm that the following document is included in the package.

- Instructions For Use 1 copy (in each box)

5. Material Provided

Catalogue No.	Description	Content (per one pack)
0023505	G11 β-Thalassemia Elution Buffer No. 1 (S)	800 mL
0023506	G11 β-Thalassemia Elution Buffer No. 2 (S)	800 mL
0023507	G11 β-Thalassemia Elution Buffer No. 3 (S)	800 mL

G11 β-Thalassemia Elution Buffers are organic acid buffers, each of which contains less than 0.05 % sodium azide as a preservative.

6. Materials Required but Not Provided

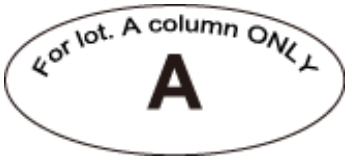
The following materials are required to perform HbF and HbA_2 analysis using the HLC-723G11 β-Thalassemia Analysis Mode. They are available separately from Tosoh Corporation.

	Catalogue No.
G11 F&A2 Calibrator Set	0023508
G11 F&A2 Control Set	0023509
TSKgel G11® β-Thal.	0023504
HSi Hemolysis & Wash Solution (L)	018431L
HSi Hemolysis & Wash Solution (LL)	0019550

Only materials obtained from Tosoh Corporation should be used. Materials obtained elsewhere should not be used as substitute since assay performance is characterized based strictly on Tosoh Corporation materials.
For detailed information about these materials, contact your local Tosoh sales representative.

7. Warnings and Precautions

1) The G11 β-Thalassemia Elution Buffer No.1, No.2 and No.3 (S) are intended for *IN VITRO* DIAGNOSTIC USE ONLY.
2) The G11 β-Thalassemia Elution Buffer is designed exclusively for use in combination with the HLC-723G11 analyzer system, TSKgel G11 β-Thal. and HSi Hemolysis & Wash Solution indicated below, never in any other combination.
   - Tosoh Automated Glycohemoglobin Analyzer HLC-723G11
   - TSKgel G11 β-Thal.
   - HSi Hemolysis & Wash Solution (L), (LL)
3) Do not use this product beyond the expiry date.
4) Note that G11 β-Thalassemia Elution Buffers must be used within 90 days after opening (provided that the pack is being squeezed and capped so that the air will not enter it).
5) This Instructions For Use (IFU) must be read combined with the Operator's Manual of the HLC-723G11 β-Thalassemia Analysis Mode.
6) Carefully read the instructions contained in this IFU and those in the IFU provided with TSKgel G11 β-Thal.
7) Always use the G11 β-Thalassemia Elution Buffers in combination with the TSKgel G11 β-Thal. of the identical lot number. The column lot number is indicated by a single uppercase alphabetical character (A, B, etc.) on the label of column box. The Elution Buffer label displays an alphabetic character corresponding to the column lot number, as shown below.



8) When there is leftover buffer in the aluminium pack that is used again but is once removed from the analyzer and stored, gently squeeze the aluminium pack by hand to remove all excess air, and store it at a temperature between 4 and 30 °C firmly tightening the cap so that the air will not enter it.
9) Do not refill an aluminium pack with any leftover Elution Buffer.
10) If you use the Lot management function on the HLC-723G11, please enter the barcode information printed on the box/pack labels of Elution Buffers. Please refer to the Operator's Manual of the HLC-723G11 β-Thalassemia Analysis Mode for detailed procedure.
11) For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state and federal regulations.
12) Patient samples (clinical specimens) must always be considered as potentially infectious and should be handled using established laboratory procedures and/or institutional guidelines.

13) Any serious incident that has occurred in relation to the device should be reported to the manufacturer and the regulatory authorities in the country where the HLC-723G11 is running.

8. Storage and Stability

All unopened materials are stable until the expiry date on the label when stored at 4 to 30 °C. The expiry date is indicated on the package box and aluminium pack labels.
The G11 β-Thalassemia Elution Buffer No. 1, No. 2 and No. 3 (S) are stable for 90 days after opening when stored at 4 to 30 °C.

9. Specimen Collection and Handling

A venous whole blood sample collected in a primary tube containing EDTA is required for the analysis.
A venous blood sample should be collected aseptically. No special preparation is necessary. Blood samples collected in primary tubes containing EDTA may be stored at 25 °C for 24 hours or at 2°-8 °C for 14 days before analysis.

10. Procedure

Be sure to read the IFU included with the TSKgel G11 β-Thal.,G11 F&A2 Calibrator Set, G11 F&A2 Control Set and HSi Hemolysis & Wash Solution as well as the Operator's Manual of the HLC-723G11 β-Thalassemia Analysis Mode for detailed instructions for their preparations.

1) Column and reagent installation
Set the following column and reagent appropriately on the analyser. The column and reagent are provided ready for use.
- TSKgel G11 β-Thal.
- HSi Hemolysis & Wash Solution (L) or (LL)

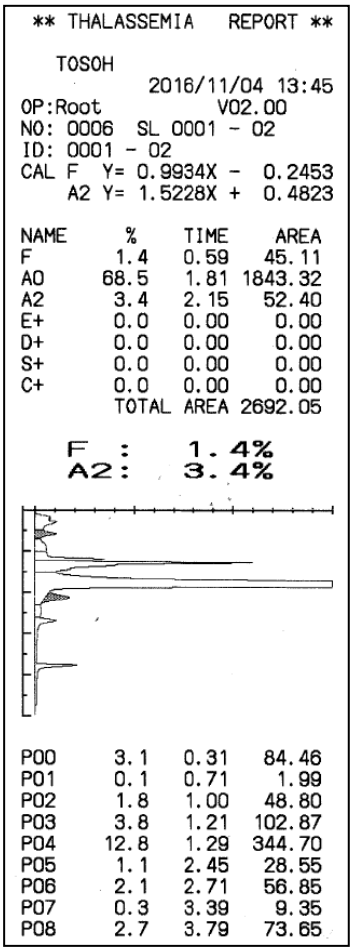
2) Buffers installation
1) Return the Elution Buffer to room temperature before use.
2) Press the "STOP" key on the HLC-723G11 system operation panel to go back to the STAND-BY state.
3) Uncap the Elution Buffer packs. Keep the caps in case the Elution Buffers are separately stored after being de-installed from the analyser.
4) Make sure to insert the tube matching the colour of the Elution Buffer pack being installed.
5) Gently squeeze the Elution Buffer pack by hand to remove all excess air inside, and firmly tighten the cap so that the air will not enter during operation.

3) Calibration
Calibration frequency should be determined based upon QC results and chromatogram quality at each laboratory. For recalibration details, please refer to the Operator's Manual of the HLC-723G11 and Instructions For Use of G11 F&A2 Calibrator Set.
The G11 F&A2 Calibrator Set is prepared to make its value traceable to the values of WHO reference reagent, NIBSC code: 89/666 on HbA_2 and to WHO reference reagent, NIBSC code: 85/616 on HbF.

4) Quality Control Procedure
In order to monitor and evaluate the precision and analytical performance, it is recommended that at least two levels of controls be run at least once per day.
G11 F&A2 Control Set is provided lyophilised. Refer to the IFU of G11 F&A2 Control Set for detailed instructions.
Assay quality control materials as instructed in the Operator's Manual of the HLC-723G11 β-Thalassemia Analysis Mode.

If one or more control samples are out of the established ranges, it is necessary to investigate the validity of the calibration curve before reporting patient results. Follow the recommendations of local, state, and federal regulatory agencies as well as laboratory policies.

A sample chromatogram for G11 F&A2 Control Set, Level (1).



** THALASSEMIA REPORT **

TOSOH

2016/11/04 13:45

OP:Root V02.00

NO: 0006 SL 0001 - 02

ID: 0001 - 02

CAL F Y= 0.9934X - 0.2453

A2 Y= 1.5228X + 0.4823

NAME	%	TIME	AREA
F	1.4	0.59	45.11
A0	68.5	1.81	1843.32
A2	3.4	2.15	52.40
E+	0.0	0.00	0.00
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	0.0	0.00	0.00
	TOTAL AREA		2692.05

F : 1.4%

A2: 3.4%

11. Results

Measurement values (%) indicate the percentage of each peak in relation to the Total Area. Note the minimum unit of measurement displayed is 0.1 %.

12. Limitations of the Procedure

1) Sample Dilution

Low concentrations of haemoglobin may cause the Total Area of the chromatogram to be < 900 in which HbF and HbA_2 values should not be reported.

High concentrations of haemoglobin may cause the Total Area of the chromatogram to be > 5000 in which HbF and HbA_2 values should not be reported.

2) Measuring Range

The measuring range of HbF is from 0.3 % to 50.0 % and HbA_2 is from 1.8 % to 9.9 %.

13. Expected Values

Reference Range (β-Thalassemia trait):

$HbA_2 \geqq 3.5$ %

Reference: National Health Service (UK). NHS Sickle Cell and Thalassemia Screening Program. November, 2006

14. Performance Characteristics

1) Dilution Linearity

A study was performed with G11 F&A2 Calibrator Level 2 diluted with various ratios of HSi Hemolysis & Wash Solution to determine linearity of HbF (%) and HbA_2 (%) values against Total Area. The results demonstrate that it is linear in samples with Total Area from 900 to 5000. It is recommended that measurement be done with Total Area from 1000 to 4500 to give more reliable results.

Total area	HbF%	HbA_2%
853	4.5	6.3
1720	4.6	6.4
2238	4.7	6.3
2789	4.7	6.4
3465	4.7	6.4
3977	4.8	6.5
4483	4.8	6.5
5318	4.8	6.5

2) Linearity

The table below shows the recovery for samples prepared by mixing two types of samples at different ratios.

High HbF sample (ratio)	Low HbF sample (ratio)	Measured value HbF (%)	Theoretical value HbF (%)	Recovery (%)
0	10	0.33	—	—
2	8	10.08	10.26	98
4	6	20.87	20.19	103
6	4	30.33	30.12	101
8	2	41.04	40.05	102
10	0	49.98	—	—

High HbA_2 sample (ratio)	Low HbA_2 sample (ratio)	Measured value HbA_2 (%)	Theoretical value HbA_2 (%)	Recovery (%)
0	10	1.76	-	-
2	8	3.22	3.38	95
4	6	4.96	5.00	99
6	4	6.54	6.62	99
8	2	8.44	8.24	102
10	0	9.86	-	-

3) Carryover

Carryover of samples was checked by measuring 2 successive blank samples set just after a sample with high Total area. Carryover (%) was calculated as:

Carryover (%) = (Total area of the blank sample) / (Total area of the sample) × 100

Total area			Carryover (%)	
Sample	Blank-1	Blank-2	Blank-1	Blank-2
7608.74	31.51	34.15	0.4	0.4

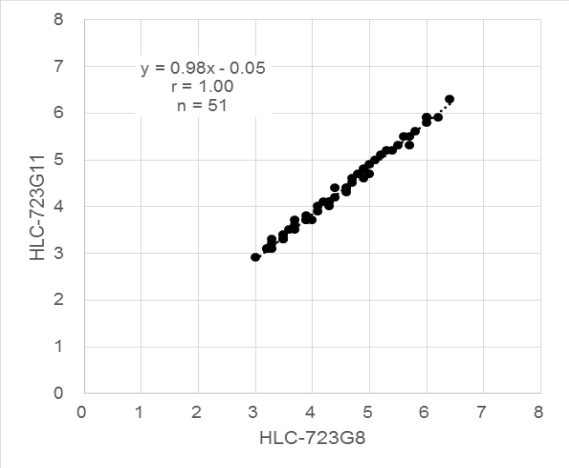
Based on these results, the carryover is not clinically significant.

4) Method comparison

A method comparison study was performed on 51 patient samples, with HbA_2 (%) values ranging from 2.9 % to 6.3 %, between the HLC-723G11 and HLC-723G8 analyzers in β-Thalassemia Analysis Mode.

The following graph illustrates the results.

Method comparison study between HLC-723G11 and HLC-723G8 analyzers.



5) Precision

1) Intra-assay precision (or within-run precision, n=20)

An intra-assay precision study (n=20) was done on 2 levels of G11 F&A2 Control Set and.

HbF

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material Level 1	1.50	0.00	0.00
Control Material Level 2	5.12	0.04	0.78

HbA_2

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material Level 1	3.19	0.03	0.94
Control Material Level 2	6.48	0.04	0.62

2) Inter-assay precision (or between-day precision, n=20)

An inter-assay precision study (n=20) was done in which 2 levels of G11 F&A2 Control Set were measured once a day for 20 operating days. The following results were obtained.

HbF

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material Level 1	1.51	0.02	1.32
Control Material Level 2	4.69	0.03	0.64

HbA_2

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material Level 1	3.17	0.06	1.89
Control Material Level 2	5.78	0.11	1.90

6) Interferences

Glucose/Triglyceride

No effect was observed below 10 g/L for glucose and 5 g/L for triglyceride.

Sodium cyanate/ Acetaldehyde

The presence of these substances may reduce the HbA_2 value. Care should be taken when interpreting alcoholic or nephritic patient specimens.

Variant haemoglobin

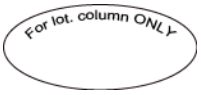
The variant haemoglobin HbE, HbD, HbS and HbC are separated from HbF and HbA_2, and HbF value and HbA_2 value are calculated. Since a high number of Hb Variants is observed worldwide, care should be taken when interpreting the results. Designation of a particular name cannot exclude the presence of other variants.

15. References

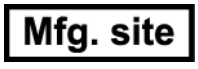
1) Bunn HF, Forget BG. Hemoglobin: Molecular, Genetic and Clinical Aspects. WB Saunders, Philadelphia 1986.
2) Huisman THJ, Carver MFH, Efremov GD. A Syllabus of Human Hemoglobin Variants. Sickle Cell Anemia Foundation, Augusta, Georgia, 1996.
3) Lehumann H, Huntsman RG. Man's Haemoglobins Including the Haemoglobinopathies and Their Investigation. North Holland Publishing Company, Amsterdam, 1974.
4) Niehuis AW, Benz EJ. Regulation of Haemoglobin Synthesis during the Development of the Red Cell. N Engl J Med 1997; 297:1318-28, 1371-81, 1430-36.
5) Stamatoyannopoulos G, Nienhuis AW. Haemoglobin switching. In: Stamatoyannopoulos G, Nienhuis AW, Majerus PW, Varmus H, eds. The Molecular Basis of Blood Diseases, pp. 107-56, 2nd ed. WB Saunders, Philadelphia, 1994.
6) Weatherall D, Clegg JB. The Thalassemia Syndromes, 3rd ed. Blackwell Scientific Publications, Oxford, 1981.

Symbols on the product labels

Net volume
(after reconstitution for lyophilised material)



For specified column lot only



Actual manufacturing site

TOSOH

TOSOH CORPORATION

BIOSCIENCE DIVISION
Shiba-Koen First Bldg.
3-8-2, Shiba, Minato-ku, Tokyo 105-8623, Japan
Phone: +81 3 5427 5181 Fax: +81 3 5427 5220

EC REP

TOSOH EUROPE N.V.

Transportstraat 4
B-3980 Tessenderlo, Belgium
Phone: +32 13 66 88 30 Fax: +32 13 66 47 49