P0502706

Tosoh Automated Glycohemoglobin Analyzer HLC-723®G11

Instructions For Use

G11® Variant Elution Buffer No. 1（S）
No. 2（S）
No. 3（S）

CE

TOSOH CORPORATION

LBL-00165

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 HbA1c 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

Symbols on the product labels

European Conformity

Manufacturer

Authorized representative in the European Community

Catalogue number / Part number

In vitro diagnostic medical device

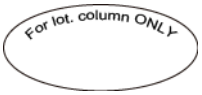
Consult instructions for use

Use-by date

/ Use by

Batch code / Lot number

Temperature limitation



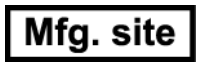
For specified

column lot only

Net volume

(after reconstitution for

lyophilized material)



Actual manufacturing site

Date of manufacture

a carrier containing unique device identifier information

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Revised in May, 2022

1. Intended Use

The G11 Variant Elution Buffers are intended for *IN VITRO* DIAGNOSTIC USE for the quantitative percent determination of hemoglobin A1c (HbA1c) in whole blood samples using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G11, Variant Analysis Mode (referred to as HLC-723G11 in this IFU), which is based on the principle of high-performance liquid chromatography assay. It is not designed for and should never be used with any other type of system. The G11 Variant Elution Buffers are intended for healthcare Professionals Use Only, in the clinical management of diabetes to assess the long-term efficacy of diabetic control in human subjects.

2. Summary and Explanation of the Test

Hemoglobin A1c measurements are used in the clinical management of diabetes to assess the long-term efficacy of diabetic control.

Glycohemoglobin (GHb) is a general term for complexes in which the whole blood glucose is non-enzymatically bound to the α or β-chains of human hemoglobin. Within these complexes, HbA1c, which is a complex of glucose and the N-terminus of the β-chain, is the most quantitatively prevalent.

HbA1c is non-enzymatically synthesized in two steps.

Step 1: The glucose aldehyde group reacts with the free amino group on the valine comprising the N-terminus of the β-chain to form the Schiff base (labile HbA1c).

Step 2: Stable ketoamine (stable HbA1c) is then formed by a reaction known as the amadori rearrangement.

Due to the fact that the labile HbA1c (the intermediate product of this reaction) changes rapidly in response to the changes in whole blood glucose concentrations, the stable HbA1c is now generally used to measure HbA1c. It provides the best indication of average glucose levels over the past couple of months because it does not fluctuate in response to physiological factors.

Abnormal hemoglobins or hemoglobin variants may co-elute with other hemoglobin components and lead to misinterpretation of HbA1c values. The Variant Analysis Mode on HLC-723G11 will produce HbA1c values that will not be affected by most commonly-known hemoglobin variants.

3. Principle of the Procedure

The HLC-723G11 is based on the principle of high-performance liquid chromatography (HPLC) with a cation exchange column, the TSKgel G11® Variant, to separate hemoglobin components into a total of six fractions in a time span of 1 minute. No pretreatment of samples

is needed.

A step gradient method is applied on the HLC-723G11, Variant Analysis Mode to separate HbA1c fraction utilizing three types of G11 Variant Elution Buffers (G11 Variant Elution Buffer No. 1, 2, and 3 (S)) with different salt concentrations. HbA1c (%) is determined as a relative percentage of the integrated area of HbA1c fraction against the sum of those of hemoglobin fractions, after being calibrated with the calibration curve established with Hemoglobin A1c Calibrator Set. or HbA1c Calibrator Set (S)

4. Before Use

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

Confirm 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)
0023479	G11 Variant Elution Buffer No. 1 (S)	800 mL
0023480	G11 Variant Elution Buffer No. 2 (S)	800 mL
0023481	G11 Variant Elution Buffer No. 3 (S)	800 mL

G11 Variant 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

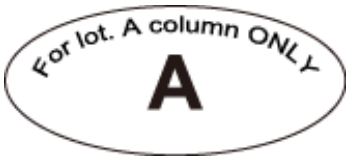
The following materials are required to perform HbA1c analysis using the HLC-723G11 Variant Analysis Mode. They are available separately from Tosoh corporation.

	Catalogue No.
Hemoglobin A1c Calibrator Set	0018767
HbA1c Calibrator Set (S)	0023502
Hemoglobin A1c Control Set	0021974
TSKgel G11 Variant	0023478
HSi Hemolysis & Wash Solution (L)	0018431
HSi Hemolysis & Wash Solution (LL)	0019550
HbA1c Diluting Solution	0023503

Only materials obtained from Tosoh Corporation should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on Tosoh Corporation materials.

7. Warnings and Precautions

1) The G11 Variant Elution Buffer No. 1, No. 2 and No. 3 (S) are for *IN VITRO* DIAGNOSTIC USE ONLY.
2) The G11 Variant Elution Buffer is designed exclusively for use in combination with the HLC-723G11 analyzer system, TSKgel G11 Variant and HSi Hemolysis & Wash Solution indicated below, never in any other combination.
   - Tosoh Automated Glycohemoglobin Analyzer HLC-723G11
   - TSKgel G11 Variant
   - HSi Hemolysis & Wash Solution (L), (LL)
3) Do not use this product beyond the expiration date.
4) Note that G11 Variant 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 Variant Analysis Mode.
6) Carefully read the instructions contained in this IFU and those in the IFU provided with TSKgel G11 Variant.
7) Always use the G11 Variant Elution Buffers in combination with the TSKgel G11 Variant 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 aluminum pack that is used again but is once removed from the analyzer and stored, gently squeeze the alminum 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 aluminum 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 Variant 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 expiration date on the label when stored at 4 to 30 °C. The expiration date indicated on the package box and aluminum pack labels.
The G11 Variant Elution Buffer No. 1, No. 2 and No. 3 (S) are stable for 90 days after opening when stored at 4 to 30 °C.
After opening, even when stored as stated above, they cannot be used beyond their expiration date.

9. Specimen Collection and Handling

A venous whole blood sample collected in a primary tube containing K2EDTA 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 K2EDTA may be stored at 25 °C for 24 hours or at 4 °C for 14 days prior to analysis.

10. Procedure

Be sure to read the IFUs included with the TSKgel G11 Variant, Hemoglobin A1c Calibrator Set, HbA1c Calibrator Set (S), HbA1c Diluting Solution, Hemoglobin A1c Control Set and HSi Hemolysis & Wash Solution as well as the Operator's Manual of the HLC-723G11 Variant Anlaysis Mode for detailed instructions for their preparations.

I. Column and Reagent Installation

Set the following column and reagent appropriately on the analyzer. The column and reagent are provided ready for use.

- TSKgel G11 Variant
- HSi Hemolysis & Wash Solution (L) or (LL)

II. 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 analyzer.

4) Make sure to insert the tube matching the color 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.

III. 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 Variant Analysis Mode and Instrucitons For Use of Hemoglobin A1c Calibrator Set or HbA1c Calibrator Set (S).

IV. Quality Control Procedure

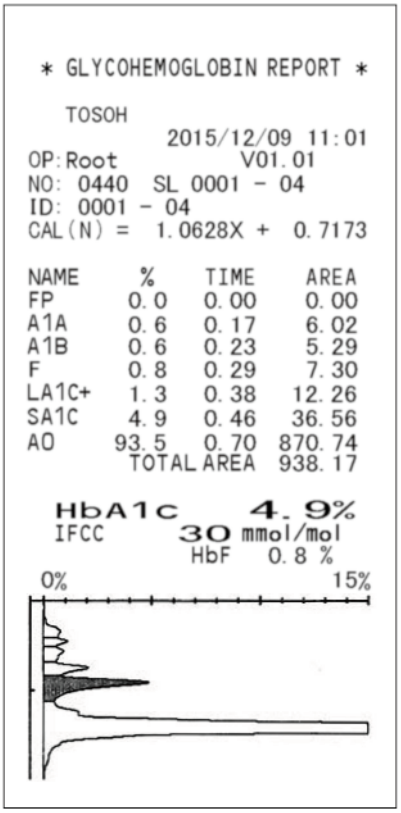
In order to monitor and evaluate the precision and analytical performance, it is recommended that at least two levels of controls be run at least once per day.

Hemoglobin A1c Control Set is provided lyophilized. Refer to the IFU of Hemoglobin A1c Control Set for detailed instructions.

Assay quality control materials as instructed in the Operator's Manual of the HLC-723G11 Variant 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 Hemoglobin A1c Control Set, Level (1).



11. Results

An HbA1c value (%) which is reported from the analyzer represents the percentage of integrated area of HbA1c fraction (“sA1c” peak shown in the above example of chromatogram) in relation to the Total Area (a total of the integrated peaks of all hemoglobin components excluding the front peak, “FP”) after calibration. HbA1c values are reported in % when the calibration is done in NGSP units, while in mmol/mol when the calibration is done in IFCC units.

Note: the minimum unit of measurement displayed is 0.1 % in NGSP units or 1 mmol/mol in IFC units.

12. Limitations of the Procedure

1) Sample Dilution

   Low concentrations of hemoglobin may cause the Total Area of the chromatogram to be < 500 in which HbA1c values should not be reported.

   High concentrations of hemoglobin may cause the Total Area of the chromatogram to be > 3500 in which HbA1c values should not be reported.

2) Abnormal Red Cell Survival

   HbA1c may not correspond to average blood glucose in patients whose red blood cell turnover is increased or decreased. Clinical interpretation has to be done with care.

3) Measuring Range

   The measuring range of HbA1c is from 2.7 % to 20.1 % in NGSP units which corresponds to 6 mmol/mol to 196 mmol/mol in IFCC units.

13. Expected Values

According to the American Diabetes Association for Caucasian (References 1) and World Health Organisation (Reference 4) :

HbA1c >/= 6.5 % threshold to diagnose diabetes

HbA1c 5.7-6.4 % categories of increased risk for diabetes (pre-diabetes)

The reference range (non-diabetic) is reported as below in Reference 2.

HbA1c : 4.0 - 6.0 % (mean 5.0 %, SD 0.5 %)

The values referred to within this IFU have been determined with a NGSP certified method. It is known that the relationship between HbA1c results from the NGSP network (%) and the IFCC network (mmol/mol) is expressed by using the following equation (Reference 3).

$$NGSP = 0.09148 \times IFCC + 2.152$$

The above categories in NGSP units can be converted to those in IFCC units below by applying the above equation.

HbA1c >/= 48 mmol/mol threshold to diagnose diabetes

HbA1c 39 – 47 mmol/mol categories of increased risk for diabetes (pre-diabetes)

The above reference range (non-diabetic) is also converted as:

HbA1c : 20 – 42 mmol/mol.

14. Performance Characteristics

I. Dilution Linearity

A study was performed with Hemoglobin A1c Control Set diluted with various ratios of HSi Hemolysis & Wash Solution to determine linearity of HbA1c (%) values against Total Area. The results demonstrate that it is linear in samples with Total Area from 500 to 3500. It is recommended that measurement be done with Total Area from 600 to 3000 to give more reliable results.

Total Area	HbA1c (%)
418	9.8
514	9.8
645	9.8
1100	9.7
1568	9.7
2009	9.8
2498	9.7
2922	9.7
3172	9.7
3325	9.7
3669	9.7

II. Recovery

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

High HbA1c sample (ratio)	Low HbA1c sample (ratio)	Theoretical value HbA1c (%)	Measured value HbA1c (%)	Recovery (%)
0	10	–	2.7	–
2	8	6.2	6.4	103
4	6	9.6	9.9	103
6	4	13.1	13.3	102
8	2	16.6	16.6	100
10	0	–	20.1	–

III. 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
3797.18	14.33	2.41	0.4 %	0.1 %

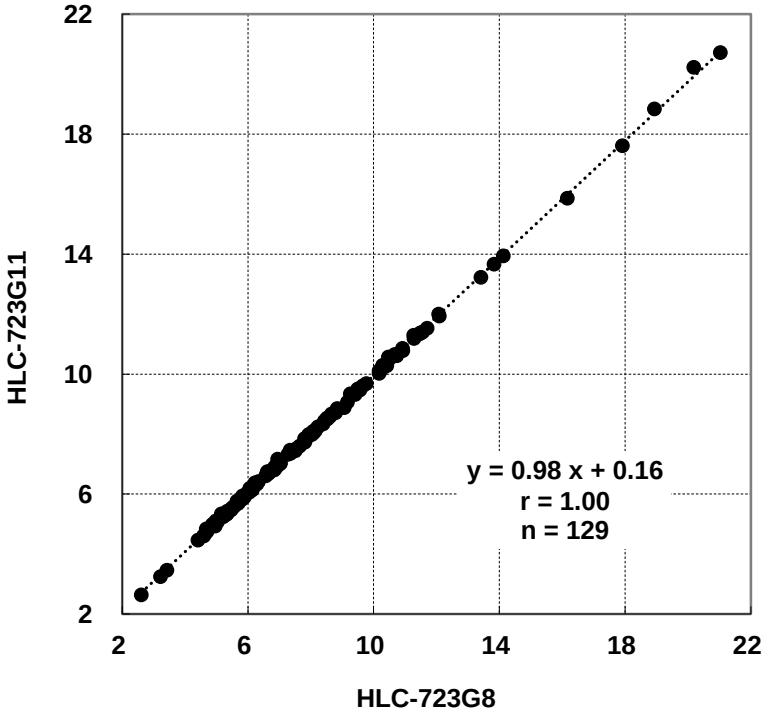
Based on these results, the carryover is not clinically significant.

IV. Method Comparison

A method comparison study was performed on 129 patient samples, with HbA1c (%) values ranging from 2.6 % to 20.2 %, between the HLC-723G11 and HLC-723G8 analyzers in Variant Analysis Mode, both of which are certified by National Glycohemoglobin Standardization Program (NGSP).

The following graph illustrates the results.

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



V. Precision

1) Intra-assay precision (or within-run precision, n=10)

An intra-assay precision study (n=10) was done on 2 levels of Hemoglobin A1c Control Set and two EDTA whole blood in NGSP units. The results were converted in IFCC units below.

(in NGSP units)

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material 5 %	4.99	0.03	0.63
Control Material 10 %	9.80	0.00	0.00
EDTA Whole blood 5 %	4.60	0.05	1.02
EDTA Whole blood 13%	13.80	0.00	0.00

(in IFCC units)

Sample	Mean (mmol/mol)	Standard Deviation (mmol/mol)	Coefficient of Variation (%)
Control Material 5 %	31.0	0.3	1.11
Control Material 10 %	83.6	0.0	0.00
EDTA Whole blood 5 %	26.8	0.5	1.92
EDTA Whole blood 13%	127.3	0.0	0.00

2) Inter-assay precision (or between-day precision, n=10)

An inter-assay precision study (n=10) was done in which 2 levels of Hemoglobin A1c Control Set and 4 level of whole blood sample were measured once a day for a 10-day period. The following results were obtained.

(in NGSP units)

Sample	Mean (%)	Standard Deviation (%)	Coefficient of Variation (%)
Control Material 5 %	4.90	0.05	0.96
Control Material 10 %	9.83	0.05	0.49
EDTA Whole blood 5 %	5.05	0.05	1.04
EDTA Whole blood 6.5 %	6.64	0.05	0.78
EDTA Whole blood 8 %	7.62	0.04	0.55
EDTA Whole blood 11%	10.51	0.09	0.83

(in IFCC units)

Sample	Mean (mmol/mol)	Standard Deviation (mmol/mol)	Coefficient of Variation (%)
Control Material 5 %	30.1	0.5	1.71
Control Material 10 %	83.9	0.5	0.63
EDTA Whole blood 5 %	31.7	0.6	1.82
EDTA Whole blood 6.5 %	49.1	0.6	1.15
EDTA Whole blood 8 %	59.8	0.5	0.77
EDTA Whole blood 11%	91.4	1.0	1.05

VI. Interferences

A substance is considered to interfere when the recovery of a known specimen falls outside a 10 % acceptability range.

1) No effect was observed below 10 g/L for glucose, 0.25 g/L for sodium cyanate and 0.25 g/L for acetaldehyde.
2) No effect was observed for the following agents up to the concentrations indicated in the table.

Free bilirubin	0.20 g/L
Conjugated bilirubin	0.20 g/L
Triglyceride	5 g/L
Ascorbic acid	2.5 g/L
Aspirin	0.50 g/L

3) Hemoglobin variants and Thalassemias
   a. The presence of HbD, HbS, HbC or HbE in the heterozygous state (such as HbAS) does not interfere with the HbA1c result.
   b. For patients with the conditions as described below, the HbA1c values should not be reported.
      - HbD, HbS, HbC or HbE is present in homozygous or double-heterozygous state (such as HbSS or HbSC)
      - Other hemoglobin variants and Thalassemias
4) Hemolysed samples do not interfere with HbA1c assay results.
5) High HbF values higher than 30 % may interfere with the HbA1c assay results.
6) HbA1c may not correspond to average blood glucose in patients whose red blood cell turnover is increased or decreased. Clinical interpretation has to be done with care.

15. References

1) American Diabetes Association. Standards of Medical Care in Diabetes - 2013. Diabetes Care 2013; 36 (Suppl. 1), S11-66.
2) American Diabetes Association. Standards of Medical Care for Patients with Diabetes Mellitus. Diabetes Care 2002; 25 (Suppl. 1), S33-49.
3) Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson J-O, Little R, Siebelder C and Weykamp C, Statistical Methods for Monitoring the Relationship between the IFCC Reference Measurement Procedure for Hemoglobin A_{1c} and the Designated Comparison Methods in the United States, Japan, and Sweden. Clinical Chemistry, 54:8, 1379-1385 (2008).
4) World Health Organization. Abbreviated Report of a WHO Consultation: WHO; 2011. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus.

TOSOH

製造販売元

東ソー株式会社

バイオサイエンス事業部

東京都港区芝 3-8-2　〒105-8623

TEL (03) 5427-5181　FAX (03) 5427-5220

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

Printed in Japan

Document Detail

Type: LBL

Document No.: LBL-00165[1]

Title: G11 Variant Elution Buffer No. 1, No. 2, No. 3 IFU

Owner: JOE.TROCHE Joseph Troche

Status: DRAFT

Effective Date:

Expiration Date:

Revision Notes

Document Build No.	Access Activity	Accessed By	Accessed Date
1	Check In	JOE.TROCHE	07-Dec-2022
Note:			
2	Check In	JOE.TROCHE	15-Dec-2022
Note:			