WELDONBIOTECH

Inspiration for life science

Wel- Chem *Direct Enzymatic HbA1c Assay*

Configuration

The Wel-Chem Direct Enzymatic HbA1c reagent is provided in the following kit configurations:

Instrument	Catalog No.	Kit size (64 ml)
Universal	HB1-149WB	Lysis Buffer 1 x 30 mL R1A: 1 x 16.8 mL R1B: 1 x 7.2 mL R2: 1 x 10 mL

Calibrators Sold Separately

Intended Use

Wel-Chem Direct Enzymatic Hemoglobin A1c (glycated hemoglobin A1c; A1c; HbA1c) reagents are intended for use in the quantitative determination of stable HbA1c in human whole blood samples. Measurement of hemoglobin A1c is a valuable indicator for long-term diabetic control. For *in-vitro* diagnostic use only.

Clinical Significance

Hemoglobin A1c is an important test recommended by the American Diabetes Association (ADA) and its usefulness was clarified by the United Kingdom Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT). Currently, the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient Diabetes management program. Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of dia-betes complications.[1-3] Therefore, the HbA1c test is a good indica-tor of glycemic control in the preceding 2-3 months.

Assay Principle

Direct Enzymatic HbA1c test is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion with Bacillus *sp* protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in *E. coli*. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide. This, in turn, is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromogen. No separate measurement for total Hemoglobin (Hb) is needed in this Direct Enzymatic HbA1c Assay.

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c.

Reagent Composition

	Concentration
Lysis Buffer	
CHES, pH 8.7	100 mM
Triton-X-100	1 %
SDS	0.45 %
Redox Agents	0.5 mM
Reagent R1A	
MES pH 7.0	5 mM
Proteases	4 KU/mL
Triton-X-100	0.5%
Redox agents	>10µM
Reagent R1B	
MES pH 6.3	1 mM
Redox agent	<3 mM
Reagent R2	
Tris pH 8.0	15 mM
FVO enzyme	>10 U/mL
POD	90 U/mL
Chromogen	0.8 mM

Materials required but not provided

1) HbA1c calibrator set : Intended for use only with Direct Enzymatic HbA1c Assay reagents
2) HbA1c calibrator set: Intended for use only with Direct Enzymatic HbA1c Assay reagents on the Co-bas Mira on-board lysis application.
3) Bi-level HbA1c controls: Whole blood hemolysates and stabilizers.
4) Direct Enzymatic HbA1c Assay blank solution: Intended for use with Direct Enzymatic HbA1c Assay reagents with instruments that require a zero calibrator.

Reagent Preparation

For analyzers capable of handling 3-reagents, R1A, R1B, R2 are ready to use. For analyzers capable of handling only 2-reagents, Weldon HbA1c reagents R1A and R1B should be mixed in a 7:3 ratio and allowed to sit at 2-8ºC for overnight prior to use. To pre-pare sufficient R1AB mixture, pour the entire contents of R1B bottle into R1A bottle. Mix gently by inversion.

Reagent Stability and Storage

Reagents are stable until their expiration date when stored at 2-8°C. Reconstituted R1AB thus prepared is stable for 4 weeks when stored at 2-8°C. **R1B and R2 reagents are light sensitive.**

Specimen Collection and Handling

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated.[4] Per CLSI guideline, it is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific specimen stability criteria for its laboratory.[12] Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes

Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.

Precautions

1) Reagent R1B and R2 are light-sensitive. Store in a dark place.
2) Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Bio-medical Laboratories (HHS Publication Number [CDC] 93-8395).
3) As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4) Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5) Do not use the reagents after the expiration date labeled on the outer box.
6) Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

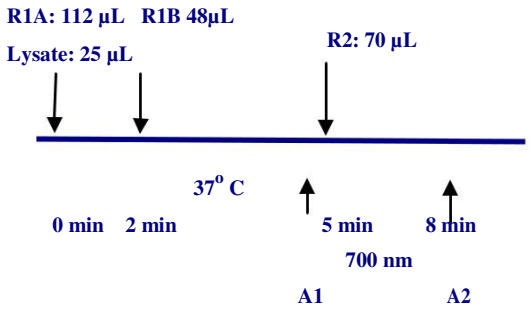
Assay Procedure

Whole Blood Bench Top Lysis Procedure

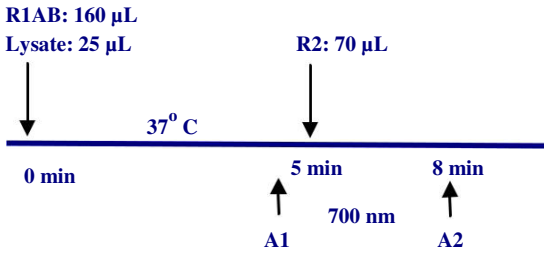
1) Dispense 250 µL of lysis buffer in a sample cup or an Eppendorf microfuge tube.
2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing.** Add 20 µL of fully resuspended whole blood sample to the lysis buffer in the sample cup or microfuge tube. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 10 min to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Enzymatic HbA1c Assay steps and is stable up to 4 hours at room temperature.
3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.

Assay Scheme for Analyzers

For analyzers capable of handling 3-reagents, please use the following scheme as a guideline for analyzer application. Note: HbA1c is an end-point assay and the first reading point A1 is right before the addition of R2.



For analyzers capable of handling only 2-reagents, please premix R1A and R1B as described in reagent preparation section and use the following scheme as a guideline for analyzer application. Note: HbA1c is an end-point assay and the first reading point A1 is right before the addition of reagent R2.



Calibration

The Weldon Direct Enzymatic HbA1c Assay requires weekly (168 hours) calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification sheet.

Weldon Direct Enzymatic HbA1c calibrator sets are intended for use with Direct Enzymatic HbA1c Assay reagents). All calibrator vials are stable until their expiration date when stored at 2-8°C. Weldon HbA1c calibrator set is in lyophilized form. Weldon HbA1c calibrator set for the Cobas Mira On-Board Lysis Application includes four levels of calibrator material. Level 0 is in liquid form and ready to use, levels 1-3 are in lyophilized form. Reconstitute lyophilized contents per instructions on labeling and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconsti-tuted calibrators are stable for 14 days when capped tightly and stored at 2-8°C. The liquid form calibrator zero is stable for 14 days after opening the vial when capped tightly and stored at 2-8°C.

Quality Control

Weldon Direct Enzymatic HbA1c control set can be purchased separately. Users should follow the appropriate feder-al, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

To ensure adequate quality control, level 1 and level 2 controls with known values should be run as unknown samples.

Results

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c. The values reported are aligned with the Diabetes Control and Complications Trial (DCCT) system and hence reported in the NGSP[8] format. No calculation step is needed.

The relationship between HbA1c results from the NGSP network (%HbA1c) and the IFCC network (mmol/mol) has been evaluated and a master equation has been developed[8, 10, 11]: NGSP = [0.09148 x IFCC] + 2.152.

Reference Range

Non-diabetic individuals have HbA1c values in the range of 3-6% and controlled diabetic individuals have HbA1c values in the 6-9% range.[9] Individuals with uncontrolled diabetes can have HbA1c as high as 20%. The American Diabetes Association (ADA) recom-

mends that the primary treatment goal in diabetes should be glucose control equal to that achieved during the DCCT.[5] Based on DCCT, ADA states HbA1c targets of <7%.[5-7] However, each laboratory must establish its own normal range in their country of business taking into account sex, age and ethnicity.

Limitations

- The linearity of the assay is up to 12% HbA1c. Samples with values above 12% should not be diluted and retested. Instead the values should be reported as higher than 12% (>12%).
- The assay is formulated for use with human whole blood samples in EDTA. Total hemoglobin in the sample should be in the range: 9-21 g/dL
- High HbF (>10%) may result in inaccurate HbA1c values.

Performance Characteristics

(Determined on Hitachi 917 chemistry analyzer)

Accuracy

The following HbA1c value data were obtained by comparing Direct Enzymatic HbA1c Assay to a legally marketed HPLC method.

	Whole blood application
n	44
Slope	1.0212
Intercept	0.0135
Correlation coefficient	0.9874
Range of values	5% - 13% HbA1c

Precision

Precision studies were conducted with the Weldon Direct Enzymatic HbA1c Assay reagents. Within-run and total precision studies were done by testing 2 levels of samples per NCCLS EP-5 procedure. Precision data is summarized in the table below:

	Level 1 (%HbA1c)	Level 2 (%HbA1c)
Mean value	5.7%	10.3%
Within run SD (Swr)	0.06	0.07
Within run CV%	1.0%	0.7%
Inter assay precision	0.10	0.18
Inter assay precision	1.8%	1.8%

Linearity

Weldon HbA1c Assay has a linear range from 4.0% - 12.0%.

Interference

The assay is not affected by the following interfering substances at the indicated concentrations: ascorbic acid 12 mg/dL, total bilirubin 15mg/dL, bilirubin (conjugated) 13mg/dL, glucose 4000mg/dL, triglyceride 4000mg/dl, uric acid 30 mg/dL, urea 80mg/dL. Stable glycated hemoglobin serves as a substrate for enzymatic reac-tion used in the Weldon Direct Enzymatic HbA1c Assay. Acetylated, carbamylated and labile HbA1c does not adversely affect the enzymatic reaction used in this assay. Variant hemoglobin S, C and E do not significantly interfere with Weldon Direct Enzymatic HbA1c Assay.

References

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