Catalog #80300

V.4

Crystal Chem

Rat Hemoglobin A1c (HbA1c) Kit Instructions

For the quantitative determination of hemoglobin A1c (HbA1c) in rat whole blood

Catalog #80300
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
1536 Brook Drive, Suite A
Downers Grove, IL 60515, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
URL: http://www.crystalchem.com

Catalog #80300

TABLE OF CONTENTS

A. Intended Use

The Rat Hemoglobin A1c (HbA1c) kit is for the quantitative determination of hemoglobin A1c (HbA1c) in rat whole blood. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Hemoglobin A1c is an important test recommended for patients with diabetes every 2-3 months as part of the patient's 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 diabetes complications. Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

Recent increases in the incidence of diabetes and obesity have stimulated intensive research on HbA1c levels. As a result, the accurate measurement of HbA1c in experimental animals is becoming increasingly important.

C. Principle of the Assay

The Rat Hemoglobin A1c (HbA1c) kit is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific fructosyl valine oxidase (FVO) enzyme. The 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 chromagen. No separate measurement for total Hemoglobin (Hb) is needed in this direct enzymatic HbA1c assay.

D. Kit Storage

1. Upon receipt of the Rat Hemoglobin A1c (HbA1c) kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
LB	Lysis Buffer (Liquid)	1 X 30 mL
CC1a	Reagent CC1a (Liquid)	1 X 16.8 mL
CC1b	Reagent CC1b (Liquid)	1 X 7.2 mL
CC2	Reagent CC2 (Liquid)	1 X 10 mL
CAL1	Calibrator 1 (lyophilized)	1 X 0.5 mL
CAL2	Calibrator 2 (lyophilized)	1 X 0.5 mL

E.2. Materials required but not provided

Microplates
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
Incubator (37ºC)
Vortex
Distilled water
Microplate reader or spectrophotometer (should read A_{700} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagent CC1b and CC2 are light-sensitive. Store in a dark place.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Venous rat 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. If assay is to be performed more than 2 weeks after collection, samples should be frozen.

I. Assay Procedure

I.1. Preparation of reagents

All reagents are provided ready-to-use and should be brought to room temperature for at least 30 minutes prior to use. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling

I.2. Preparation of samples, calibrators, and controls

1. Reconstitute the calibrators with 0.5 mL distilled water. To ensure complete reconstitution, equilibrate vials at room temperature for 30 minutes before first use.
   Note: Reconstituted calibrators are stable for 14 days when capped tightly and stored at 2-8°C.
2. Bring all samples, calibrators, and controls to room temperature. Frozen samples should be allowed to fully thaw before proceeding.

3. Prior to testing, whole blood samples, calibrators, and controls should be thoroughly mixed by gentle inversion.
   Note: ***Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing.*** *Whole blood samples should be mixed at least 5 times by gentle inversion to resuspend settled erythrocytes.*

I.3. Preparation of lysate

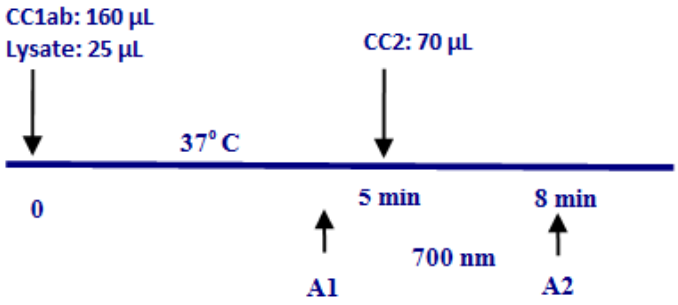
1. Dispense 62.5 µL of Lysis Buffer (marked "LB") into a clean glass tube.
2. Then dispense 5 µL of fully resuspended whole blood sample, calibrator, or control into the glass tube.
3. Mix gently with pipette without creating foam.
4. Mix hemolysate by vortex and incubate at room temperature (25°C) for 10 mins 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. It is important that all small clots are fully dissolved in the lysis buffer. The lysate, thus prepared, is ready for use and is stable up to 4 hours at room temperature.
5. Repeat steps 1-4 for all samples, calibrators, and controls.

I.4. Assay procedure

The procedure below reflects a manual procedure performed using a microplate and a microplate reader (ideal when running multiple samples). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers. Please contact Crystal Chem for more information.

1. Add 112 µL of Reagent CC1a and 48 µL of Reagent CC1b into each well (as needed) of a microplate and mix well by repeated pipetting. Avoid bubbles.
2. In each well, add 25 µL lysate of sample, calibrator, or control and mix well by repeated pipetting.
3. Place microplate in incubator (37ºC) and allow microplate to equilibrate to 37ºC over 5 minutes.
4. Measure absorbance using a plate reader (measure A_{700} values).
   Note: The Rat HbA1c assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC2.
5. Add 70 µL of Reagent CC2 and mix well by repeated pipetting.
6. Measure the increase in absorbance after 3 minutes at 37ºC using a plate reader (measure A_{700} values).

Figure 1. Summary of assay procedure



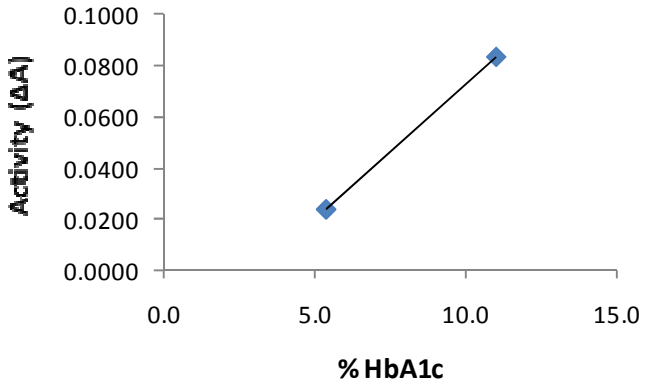
I.5. Determining the HbA1c concentration

1. Calculate the change in absorbance ΔA (0sec ~ 180sec)

   $\Delta A = (OD_{700nm,\ 180sec}) - (OD_{700nm,\ 0sec}) \times 185/255$

2. Using linear graph paper, construct the HbA1c calibration curve by plotting the mean change in absorbance value for each calibrator on the Y axis versus the corresponding HbA1c concentration on the X axis.
   Note: *Calibrator values vary per lot and should be obtained from the calibrator labels. A calibration curve should be plotted every time the assay is performed.*
3. Rat HbA1c concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. 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.

Figure 2. Typical calibration curve (linear fit)



J. Performance characteristics

J.1. Assay range

The Rat Hemoglobin A1c (HbA1c) assay has a linear range from 3.5% - 13.0%.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.