HbA1c Assay Kit

ARG83563 HbA1c Assay Kit can be used to measure HbA1c in Whole blood, other biological fluids

Catalog number: ARG83563

Package: 96 wells

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MANUFACTURED BY:

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PRINCIPLE OF THE ASSAY

ARG83563 HbA1c Assay Kit t provides an accurate, convenient measure of Hemoglobin A1c concentration in biological fluids such as whole blood and other biological fluids. In the assay, the denatured whole blood sample is decomposed by protease to amino acids, including valine on the glycated hemoglobin β chain. The fructovaline oxidase react with glycated valine and produces H2O2, which is coupled to the corresponding chromogen. The concentration can be measured at 570 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Enzyme I, Enzyme II and Dye Reagent at-20 °C and protect from light, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4 °C
Hemolytic Agent	10 ml	4 °C
Enzyme I	1 vial (lyophilized)	-20 °C (protect from light)
Enzyme II	1 vial (lyophilized)	-20 °C (protect from light)
Reaction Buffer	10 ml	4 °C
Dye Reagent	1 vial (lyophilized)	-20 °C (protect from light)
Plate sealer	3 adhesive strips	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Enzyme I, Enzyme II and Dye Reagent at-20 °C and protect from light, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Whole blood - EDTA anticoagulant whole blood, refrigerated at 2°C- 8°C can be stable for 24-36 hours, mixed before use. Mix 10 µl whole blood with 90 µl hemolytic agent to avoid foaming, incubate at room temperature for 15-20 minutes, gently mix several times during incubation. When the mixture becomes a clear, dark red liquid, it proves that the whole blood has been completely dissolved. The samples after hemolysis should be tested on the same day, and the room temperature can be stable for 4 hours.

REAGENT PREPARATION

- **Enzyme I:** Reconstitute the **Enzyme I** with **8 ml** of Reaction Buffer. Allow the **Enzyme I** keep on bench for few minutes. Make sure the **Enzyme I** is dissolved completely and mixed thoroughly before use.

- **Enzyme II:** Reconstitute the **Enzyme II** with **1 ml** of Reaction Buffer. Allow the **Enzyme II** keep on bench for few minutes. Make sure the **Enzyme II** is dissolved completely and mixed thoroughly before use.

- **Dye Reagent:** Reconstitute the **Dye Reagent** with **10 ml** of distilled water. Allow the **Dye Reagent** keep on bench for few minutes. Make sure the **Dye Reagent** is dissolved completely and mixed thoroughly before use.

- **Standard:** Reconstitute the **Standard** with 1 ml of distilled water. Allow the **Standard** keep on bench for few minutes, to yield 5 µmol/ml **standard stock**. Make sure the **Standard** is dissolved completely. Perform 2-fold serial dilutions of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Summary of HbA1c Assay Kit Procedure

Reagent	Standard	Blank	Sample
Standard	10 µl	-	-
Distilled water	-	10 µl	-
Sample	-	-	10 µl
Enzyme I	80 µl	80 µl	80 µl
Mix well incubate for 15 min at 37 °C			
Enzyme II	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix well incubate for 15 min at 37 °C Read the OD at 570 nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition:

$C_{Standard}$: the standard concentration, 5 µmol/ml =5 mmol/L;

$V_{Standard}$:the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml.

n: dilution factor, n = 10.

b.) Calculation:

Formula:

a). According to the protein concentration

HbA1c (mmol/L) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}] \times n$

$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$