Hemoglobin A1c Microplate Assay Kit

User Manual

Catalog # CAK1305

(Version 1.1A)

Detection and Quantification of Hemoglobin A1c (HbA1c) Content in Whole blood and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Creatine is present in vertebrates and helps to supply energy to muscle. In humans and animals, approximately half of creatine originates from food (mainly from fresh meat). Creatine supplementation has been investigated as a possible therapeutic approach for the treatment of muscular, neuromuscular, neurological and neurodegenerative diseases.

Hemoglobin A1c Microplate Assay Kit 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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Hemolytic Agent	10 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Enzyme I	Powder x 1	-20 °C, keep in dark
Enzyme II	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme I: add 8 ml Reaction Buffer to dissolve before use.

Enzyme II: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent: add 10 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use. The concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For whole blood samples

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.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Standard	Blank	Sample
Standard	10 µl	--	--
Distilled water	--	10 µl	--
Sample	--	--	10 µl
Enzyme I	80 µl	80 µl	80 µl
Mix, put it in the oven, 37 °C for 15 minutes.			
Enzyme II	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it in the oven, 37 °C for 15 minutes, measured at 570 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$HbA1c\ (mmol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

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

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

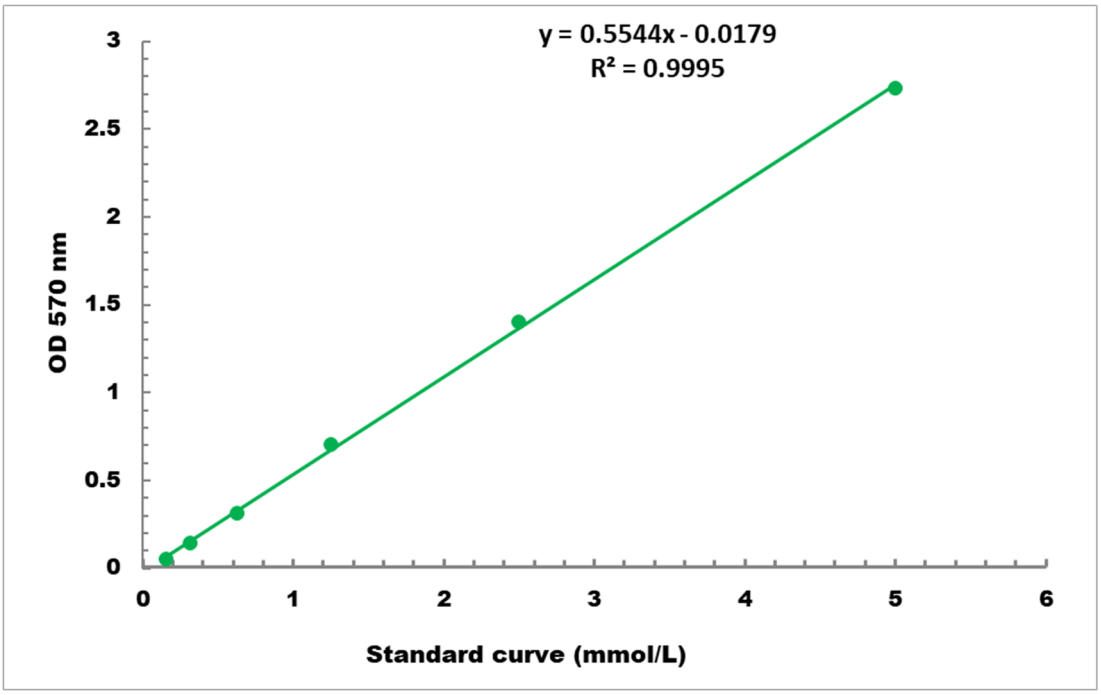
$V_{Standard}$: the volume of standard, 0.01 ml;

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

n: dilution factor, n = 10.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES