Hemoglobin A1c

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1305

(Version 1.2A)

Detection and Quantification of Hemoglobin A1c (HbA1c)Content inWhole blood and Other biological fluidsSamples.

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

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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 investigatedas a possible therapeutic approach for the treatment of muscular,neuromuscular, neurological and neurodegenerative diseases.

Hemoglobin A1cColorimetric Microplate Assay Kit provides an accurate, convenient measure of Hemoglobin A1cconcentration in biological fluids such as whole blood andother 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 570nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Hemolytic Agent	10 mlx 1	4 °C
Reaction Buffer	10mlx 1	4 °C
Enzyme I	Powderx 1	-20 °C, keep in dark
EnzymeII	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	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate 5 mmol/L of standardstock/top solution,store at 4 °C for 1-2 weeks or -20°C for 2-3 monthsafter reconstitution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Enzyme I: Add 8 ml Reaction Bufferto dissolve before use. Prepare for immediateuse or store at -80°C for 2-3 months after reconstitution.

Enzyme II: Briefly centrifuge prior to opening. Add 1 ml Reaction Bufferto dissolve before use. Prepare for immediateuse or store at -80°C for 2-3 months after reconstitution.

Dye Reagent: Add 10 ml distilled waterto dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.Forwhole blood samples

EDTA anticoagulant whole blood, refrigerated at 2°C- 8°C can be stable for 24-36 hours, mixed before use. Mix 10µl whole blood with 90µl 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.

VI. ASSAY PROCEDURE

Warm all reagentsto room temperature before use.

Add following reagents intothe 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 recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

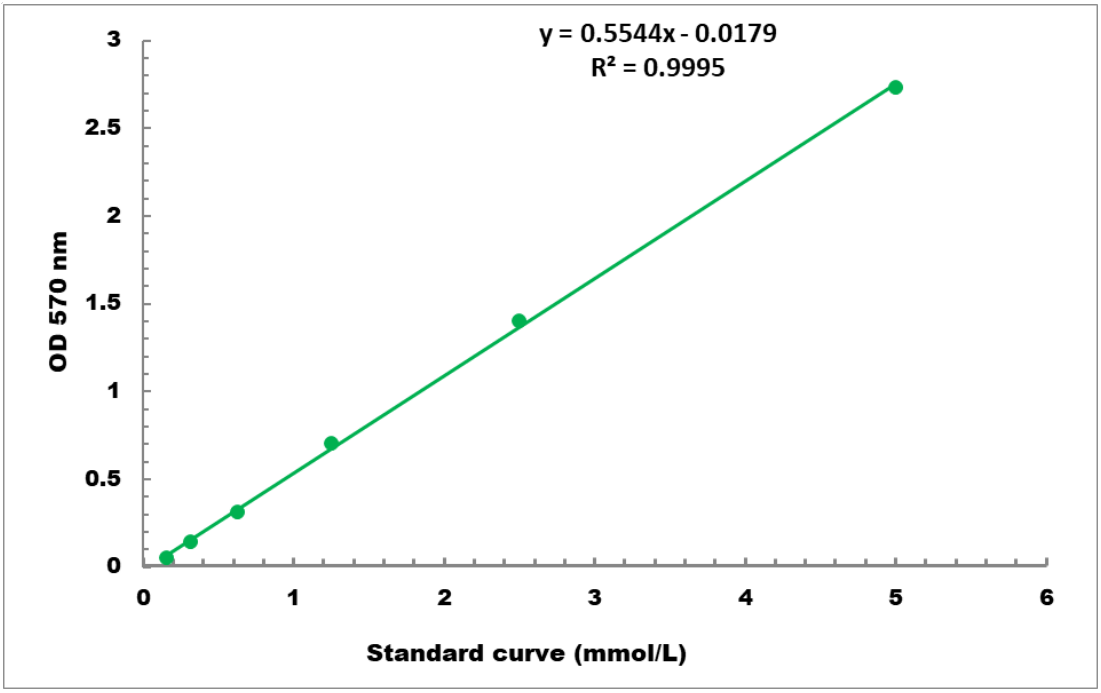
$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

VIII. 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