Reducing Sugar

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1326

(Version 1.1A)

Detection and Quantification of Reducing Sugar Concentration in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Reducing sugars are carbohydrates that act as a reducing agent with a free aldehydic (-CHO) or ketonic (-CO-) group in its structure and get oxidized by weak oxidizing agents like salts of metals. The presence of free carbon at the end of these reducing sugars is known as reducing ends. All categories of carbohydrates: monosaccharides, disaccharides, oligosaccharides, and polysaccharides include reducing sugars, whereas all monosaccharides, some disaccharides, some oligosaccharides, and some polysaccharides are reducing sugars.

Reducing Sugar Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring reducing sugar concentration in a variety of samples. The reducing sugar reacts with 3,5-dinitrosalicylic acid, and can be measured at a colorimetric readout at 540 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	20 ml x 1	4 °C
Assay Buffer II	20 ml x 1	4 °C
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
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 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 10 mmol/L stock standard solution. Store stock solution at -20 °C for 3 months. Add 0.5 ml stock solution into 0.5 ml distilled water, the concentration will be 5 mmol/L as top standard solution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312 mmol/L.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 800 µl distilled water for 5×10^6 cells or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 800 µl distilled water, transfer it into the centrifuge tube; then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

If the sample does not contain any protein or less protein, it can be assayed directly. If the sample contains more protein, the samples should be cleared by mixing 800 µl sample with 100 µl Assay Buffer I and 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection (dilution factor n = 1.25).

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	50 µl	50 µl	50 µl
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Dye Reagent	100 µl	100 µl	100 µl
Put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.			

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.

VII. CALCULATION

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

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (\mu mol/ml)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the quantity of cells

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.2 According to the weight of sample

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

2.3 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, μmol/ml

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

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

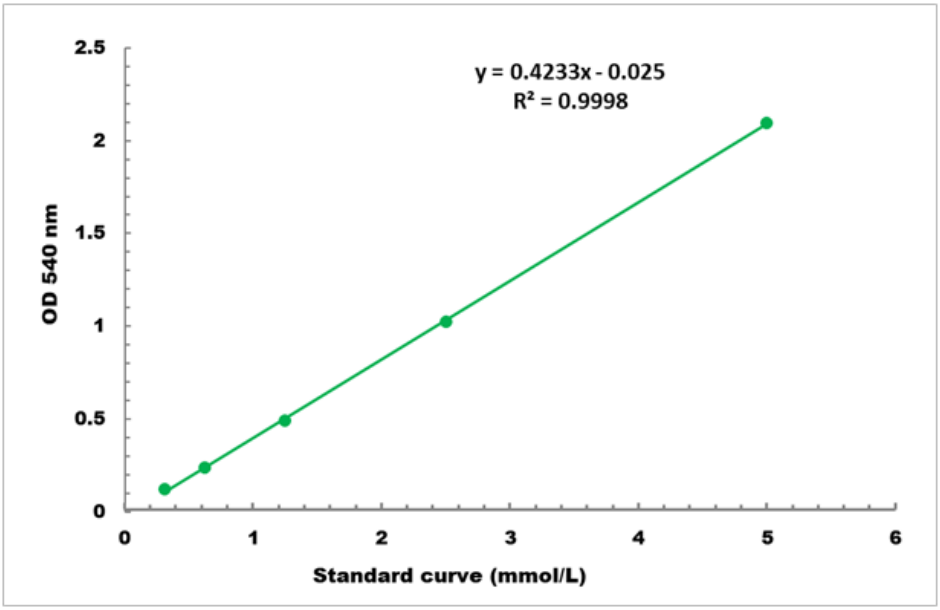
V_{Assay}: the volume of distilled water and Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell, 10^4

VIII. TYPICAL DATA

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



Detection Range: 0.5 mmol/L - 5 mmol/L