



Soluble Sugar Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1186

(Version 1.3A)

Detection and Quantification of Soluble Sugar Content in Serum,
Plasma, Cell culture, Tissue extracts, Cell lysate, Other biological
fluids/Samples.

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

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII. TYPICAL DATA.....	9

I. INTRODUCTION

Soluble sugar is an important osmotic regulator in plants and is closely related to stress resistance. The stress of plant carbohydrates on low temperature, drought and other stress conditions showed an increase in soluble carbohydrate content of plants. In addition to its important role in metabolism, soluble carbohydrates such as glucose and sucrose help regulate many developmental and physiological processes in plants.

Soluble SugarColorimetric Microplate Assay Kit is designed to directly measure soluble sugar content in a variety of samples. In the assay, soluble sugar reacts with anthrone. The product is determined at 620nm, is directly proportional to the soluble sugar concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	15 mlx 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 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulating oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 10 mg/ml of standard stock solution, store at 4 °C for 1-2 months after reconstitution. Then dilute to 200 µg/ml standard top solution by adding 20 µl stock solution into 980 µl distilled water. 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 200/100/50/25/12.5/6.25/3.12 µg/ml.

Dye Reagent Diluent: Be careful, it is strong acid.

Dye Reagent: Add 15 ml Dye Reagent Diluent to dissolve before use, keep in dark and store at 4 °C for 1 day after reconstitution.

V. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, transfer it into microcentrifuge tubes, incubate at 80°C for 30 minutes, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	50 μ l	--	--
Standard	--	50 μ l	--
Distilled water	--	--	50 μ l
Dye Reagent	150 μ l	150 μ l	150 μ l
Mix, put the microplate into the hot air circulating oven, incubate at 90°C for 15 minutes, when cold record absorbance measured at 620 nm.			

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

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

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 weight of sample

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

2.2 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard}: the standard concentration, $\mu\text{g/ml}$

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

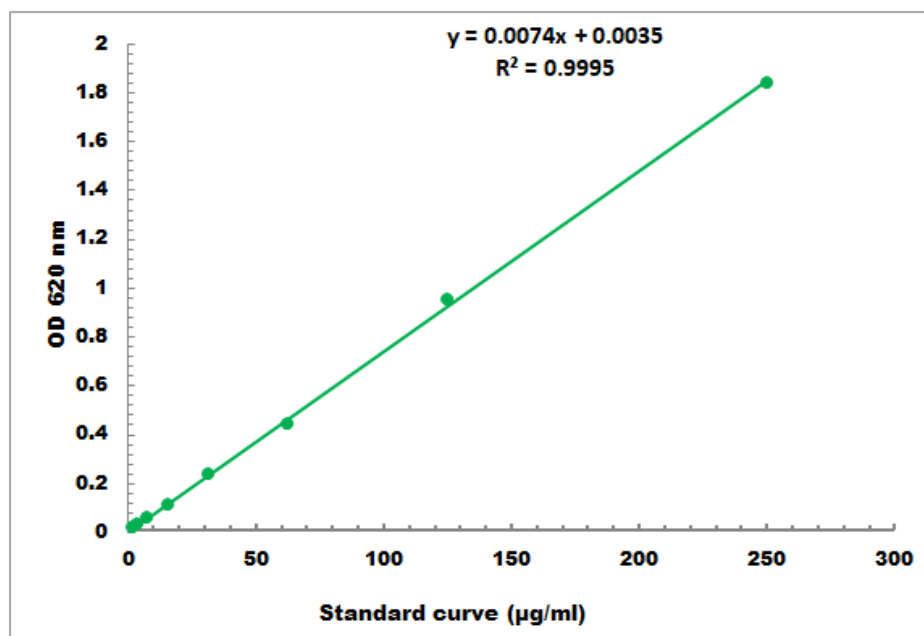
V_{Sample}: the volume of sample in assay procedure, μl

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

W: the weight of sample, g

VIII. TYPICAL DATA

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



Detection Range: 2µg/ml - 200µg/ml