Fructose Microplate Assay Kit

User Manual

Catalog # CAK1035

(Version 1.2C)

Detection and Quantification of Fructose Content in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Fructose is a monosaccharide found in many foods and is one of the three most important blood sugars along with glucose and galactose. Fructose is the sweetest naturally occurring sugar, estimated to be twice as sweet as sucrose.

Fructose is reacted with resorcinol to generate colored matter under acid conditions, and have characteristic absorption peak at 480nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Dye Reagent: add 5 ml distilled water to dissolve before use.

Standard: add 2 ml distilled water to dissolve before use, the concentration will be 1 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 480 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer, put it in water bath of 80 °C for 10 minutes, centrifuged at 4,000g at room temperature for 10 minutes, take the supernatant into a new centrifuge tube.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Reaction Buffer	100 µl	100 µl	100 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, put it into the convection oven, 90 °C for 20 minutes; when cold, record absorbance measured at 480 nm.			

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

$$\text{Fructose (mg/ml)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

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

2. According to the weight of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 1 mg/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

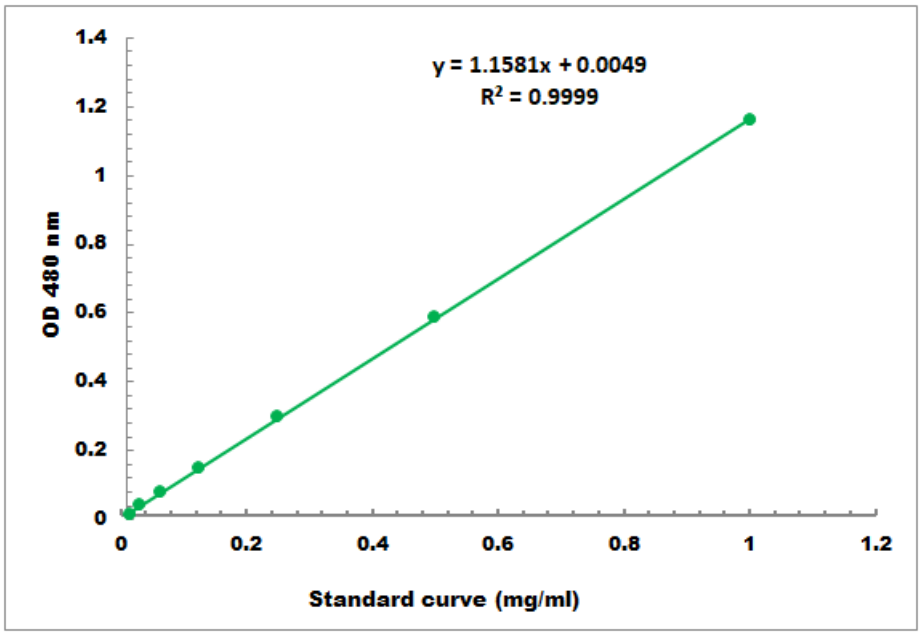
$V_{Standard}$: the volume of standard, 0.05 ml;

V_{Sample}: the volume of sample, 0.05 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 0.01 mg/ml - 1 mg/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES