Fructan Microplate Assay Kit

User Manual

Catalog # CAK1247

(Version 1.1A)

Detection and Quantification of Fructan Content in Tissue extracts, Cell lysate and Other biological fluids Samples.

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

I. INTRODUCTION

Fructan is a poly (fructose) molecule naturally produced as a storage compound in a limited number of plants and characterized by a low degree of polymerization (5-60 units). Such polymers can be hydrolyzed enzymatically or chemically to yield fructose, which is becoming an increasingly popular sweetener in many food products. Because oligofructose molecules are sweet, fructans themselves can be utilized directly as natural sweeteners. Also, the human digestive system has no enzymes that can degrade the β(2→1) or β(2→6) glycosidic linkages found in fructan, making this sugar attractive as a low-calorie food ingredient. Besides plants, microorganisms are capable of producing fructans of very high molecular weight (> 100,000 units). Fructan Microplate Assay Kit provides a convenient tool for sensitive detection of fructan concentration in a variety of samples. The assay is initiated with the enzymatic hydrolysis of fructan by Inulinase. The intensity of the product color, measured at 550 nm, is proportional to the fructan concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	Powder x 2	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme I: add 1 ml Reaction Buffer to dissolve before use. Store at -80 °C for 1 month.

Enzyme II: for each tube, add 1 ml Reaction Buffer to dissolve before use. Store at -80 °C for 1 month.

Dye Reagent: add 20 ml distilled water to dissolve before use. Store at -20 °C for 1 month.

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 5 mg/ml. Store at -20 °C for 1 month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 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.

2. For liquid samples

Detect directly, or dilute with distilled water.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl	60 µl
Sample	20 µl	20 µl	--	--
Standard	--	--	20 µl	--
Distilled water	--	10 µl	--	20 µl
Enzyme I	10 µl	--	10 µl	10 µl
Enzyme II	10 µl	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 40 °C for 30 minutes, record absorbance measured at 550 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

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

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 5 mg/ml;

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

W: the weight of sample, g;

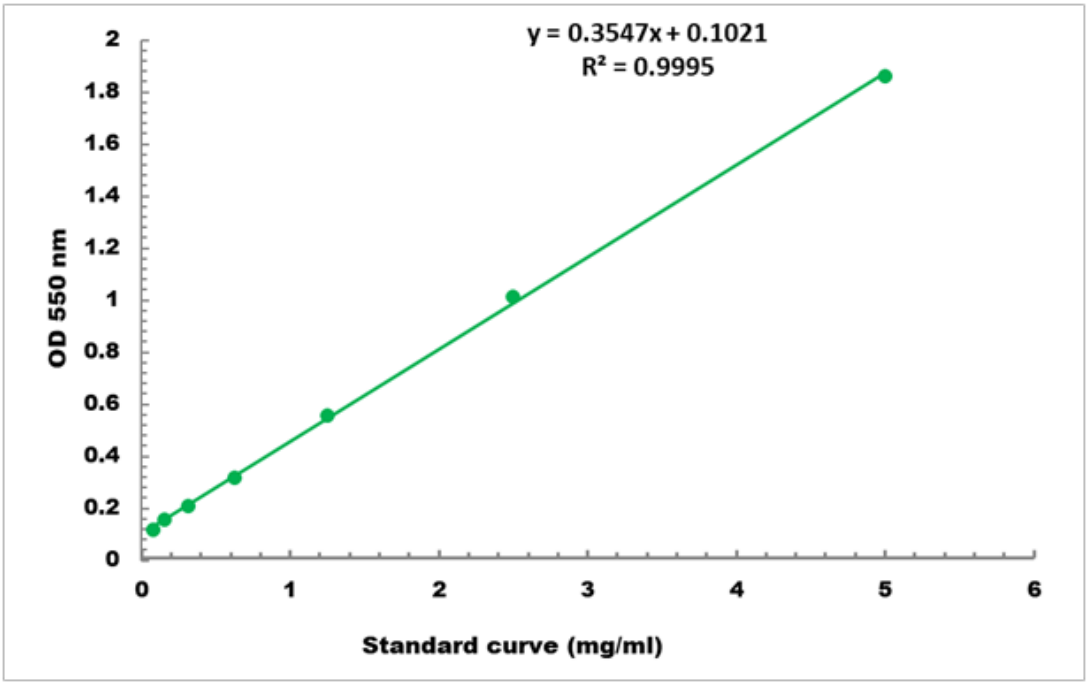
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 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: 0.05 mg/ml - 5 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