Fructan

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1247

(Version 1.2A)

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). FructanColorimetric 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 mlx 4	4 °C
Reaction Buffer	20 mlx 1	4 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	Powder x 2	-20 °C
Dye Reagent	Powder x 1	4 °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 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulation oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate5 mg/ml of standardtop solution, store at 4 °C for 2-3 weeks or -20°C for 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 be5/2.5/1.25/0.625/0.312/0.156/0.078mg/ml.

Enzyme I: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Store at -80 °C for 1 month.

Enzyme II: Briefly centrifuge prior to opening. for each tube, add 1 ml Reaction Buffer to dissolve before use. Store at 4°C for 2-3 days or -80 °C for 1 month.

Dye Reagent: Add 20 ml distilled water to 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.For tissue samples

Weigh out 0.1g 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.

VI. ASSAY PROCEDURE

Add following reagents intothe 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:

*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

$$C=\frac{(OD_{Sample}-OD_{Control})-\text{Intercept}}{\text{Slope}}\times n(mg/ml)$$

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

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

2.2According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

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

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

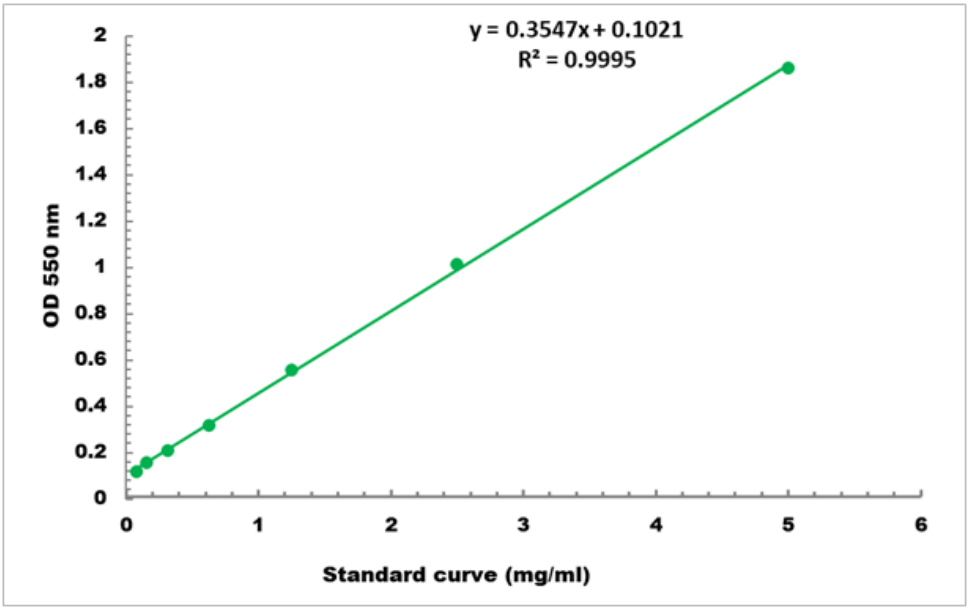
V_{Sample}: the volume of sample in assay procedure, µl

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: 0.05 mg/ml -5 mg/ml