Inulinase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1248

(Version 1.3A)

Detection and Quantification of InulinaseActivity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Inulinases are enzymes that mostly hydrolyze inulin, a polyfructan, but also levan and sucrose. Inulinases can have either endo- or exo-action. The former, endo-inulinase (EC 3.2.1.7. 2,1–β-D-fructanfructanohydrolase) catalyzes endohydrolysis of 2,1-β-D-fructosidic linkages in inulin, resulting in the formation of fructo-oligosaccharides (FOS), used as prebiotics; the latter, exo-inulinase (EC 3.2.1.80) hydrolyzes terminal, non-reducing 2,1- and 2,6-linked β-D-fructofuranose residues in fructans, and are used in the production of ultra-high fructose syrups. Fructose is sweeter than sucrose and does not crystalize easily, hence it is used as a sweetener in beverages and in confectionery.

Inulinase Activity Microplate Assay Kit provides a convenient tool for sensitive detection of inulinase activity in a variety of samples. Inulinaseshydrolyze inulinto glucose.The intensity of the product color, measured at 550 nm, is proportional to the inulinase activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	Powder x 1	4 °C
Reaction Buffer	15 mlx 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °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. Convection oven
9. Ice

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 10 mmol/L of standardtop solution.Store at 4 °C for 1 month or -20°C for 5-6 months after reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Substrate: Add 9 ml Reaction Buffer to dissolve before use.Store at 4 °C for 1 month or -20°C for 4-5months after reconstitution.

Enzyme: Briefly centrifuge prior to opening. 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 10 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.

Positive Control:Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use.Store at -80 °C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	
Positive Control	--	--	--	--	10 µl
Standard	--	--	10 µl	--	--
Distilled water	--	--	--	10 µl	--
Reaction Buffer	--	10 µl	90 µl	90 µl	--
Substrate	90 µl	90 µl	--	--	90 µl
Mix, put it into the convection oven, incubate at 40°C for 10 minutes.					
Enzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Dye Reagent	90 µl	90 µl	90 µl	90 µl	90 µl
Mix, put it in the oven,37 °C for 5 minutes, record absorbance measured at 550 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One unit ofinulinase activity is defined as the enzyme generates1 μmol of reducing sugar per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

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

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N\times(V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times(V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

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

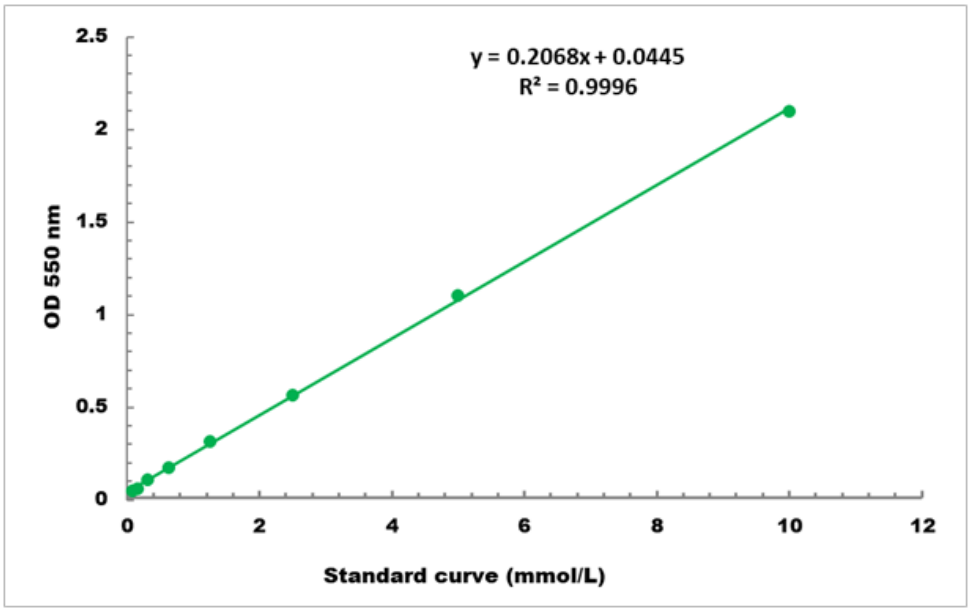
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

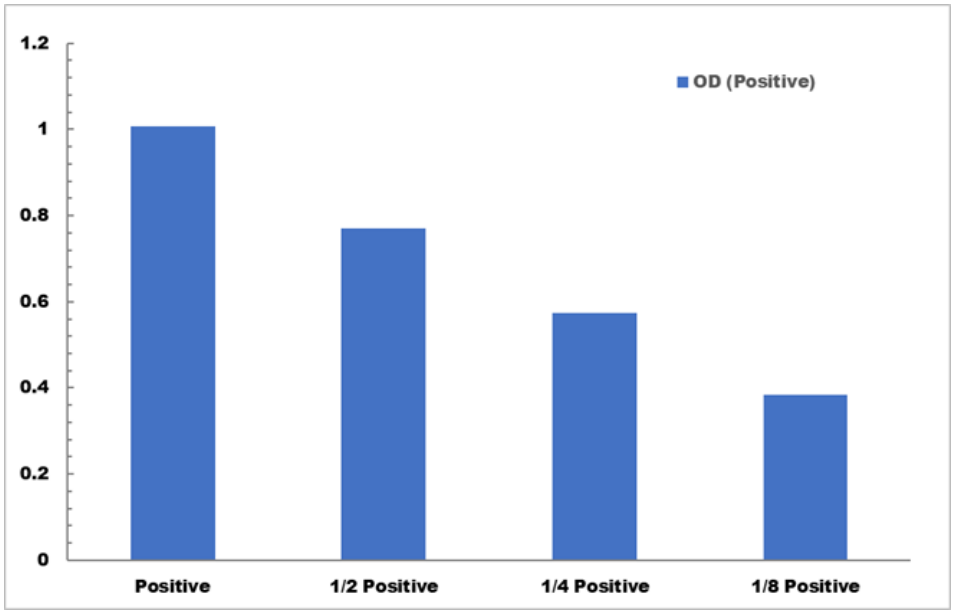
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L -10 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration