Inulinase Activity Microplate Assay Kit

User Manual

Catalog # CAK1248

(Version 1.1A)

Detection and Quantification of Inulinase Activity 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-fructan fructanohydrolase) 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. Inulinases hydrolyze inulin to 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 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Reaction Buffer	15 ml x 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	
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Note:

Substrate: add 9 ml Reaction Buffer to dissolve before use. Store at -20 °C for 1 month.

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

Dye Reagent: add 10 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 10 mmol/L. Store at -20 °C for 1 month.

Positive Control: add 1 ml Assay Buffer to dissolve before use. Store at -80 °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
9. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay 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 10 minutes, 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 ml Assay 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.

3. For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents into the 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of inulinase activity is defined as the enzyme generates 1 μmol of reducing sugar per minute.

1. According to the protein concentration of sample

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

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

2. According to the volume of sample

$$\text{Inulinase (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

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

3. According to the weight of sample

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

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

$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml;

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

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.01 ml;

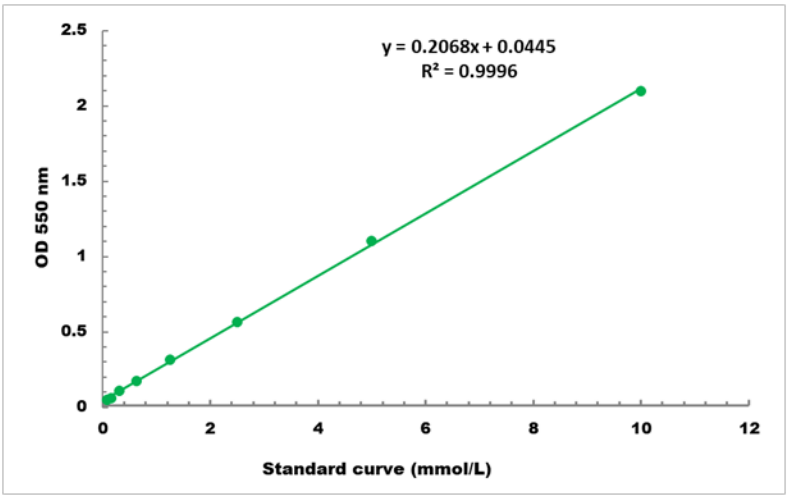
V_{Sample}: the volume of sample, 0.01 ml;

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

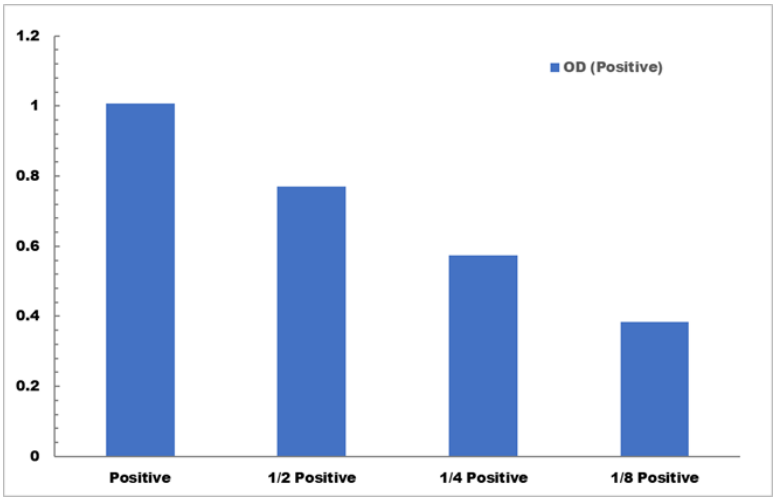
T: the reaction time, 10 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

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

IX. NOTES