Glycogen Branching Enzyme Microplate Assay Kit

User Manual

Catalog # CAK1132

(Version 1.2C)

Detection and Quantification of Glycogen Branching Enzyme 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

Glycogen branching enzyme (EC 2.4.1.18) is an enzyme that adds branches to the growing glycogen molecule during the synthesis of glycogen, a storage form of glucose. More specifically, during glycogen synthesis, a glucose 1-phosphate molecule reacts with uridine triphosphate (UTP) to become UDP-glucose, an activated form of glucose. The activated glucosyl unit of UDP-glucose is then transferred to the hydroxyl group at the C-4 of a terminal residue of glycogen to form an α-1,4-glycosidic linkage, a reaction catalyzed by glycogen synthase. Importantly, glycogen synthase can only catalyze the synthesis of α-1,4-glycosidic linkages. Since glycogen is a readily mobilized storage form of glucose, the extended glycogen polymer is branched by glycogen branching enzyme to provide glycogen breakdown enzymes, such as glycogen phosphorylase, with a large number of terminal residues for rapid degradation. Branching also importantly increases the solubility and decreases the osmotic strength of glycogen.

Glycogen branching enzyme Microplate Assay Kit is a sensitive assay for determining Glycogen branching enzyme in various samples. Glycogen branching enzyme activity is determined by iodine. The reaction products can be measured at a colorimetric readout at 660 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer I	6 ml x 1	4 °C
Reaction Buffer II	8 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Inhibitor	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Substrate: add 1 ml distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute.

Inhibitor: add 1 ml distilled water to dissolve before use, mix.

Dye Reagent: add 1 ml distilled water to dissolve before use, mix.

Standard: add 1 ml distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute; the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Water bath
8. Ice

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For liquid samples

Add 0.9 ml Assay buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	--	--	--
Distilled water	--	40 µl	20 µl	60 µl
Reaction Buffer I	50 µl	50 µl	50 µl	50 µl
Substrate	10 µl	10 µl	--	--
Inhibitor	10 µl	10 µl	--	--
Mix, 37 °C wait for 20 minutes, then put the microcentrifuge tubes into the boiling water for 1 minute, add all the reagents into the microplate.				
Reaction Buffer II	80 µl	80 µl	80 µl	80 µl
Standard	--	--	40 µl	--
Dye Reagent	10 µl	10 µl	10 µl	10 µl
Mix, wait for 10 minutes, measured at 660 nm and record the absorbance.				

Note:

1) Substrate and Standard should be mixed well before adding to the microcentrifuge tubes and plate.

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

3) 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 Glycogen Branching Enzyme activity is defined as the enzyme decompose 1 μmol soluble starch per minute.

1. According to the protein concentration of sample

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

$$= 0.25 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.25 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$GBE\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T \times n$$

$$= 2.5 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 5 mmol/L = 5 μmol/ml;

$V_{Standard}$: the volume of standard, 40 μl = 0.04 ml;

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

W: the weight of sample, g;

V_{Sample}: the volume of sample, 40 μl = 0.04 ml;

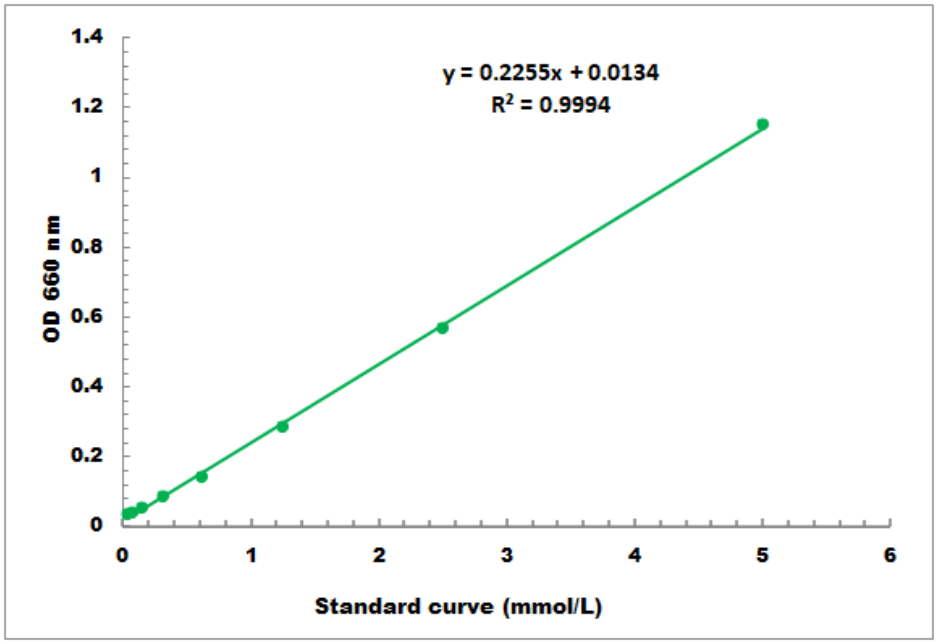
V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 20 minutes;

n: dilution factor = 10

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES