Glycogen Branching Enzyme Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1132

(Version 1.3C)

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 Activity Colorimetric 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
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water, mix and heat in boiling water bath for 1 minute to generate 5 mmol/L of standard top solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute. Store at 4 °C for 1 day or -20°C for 3 days after reconstitution.

Inhibitor: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix. Store at -20°C for 3-4 months after reconstitution.

Dye Reagent: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix. Keep in dark and store at 4 °C for 1-2 months after reconstitution.

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

V. 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.

VI. 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:

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

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

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

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

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

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

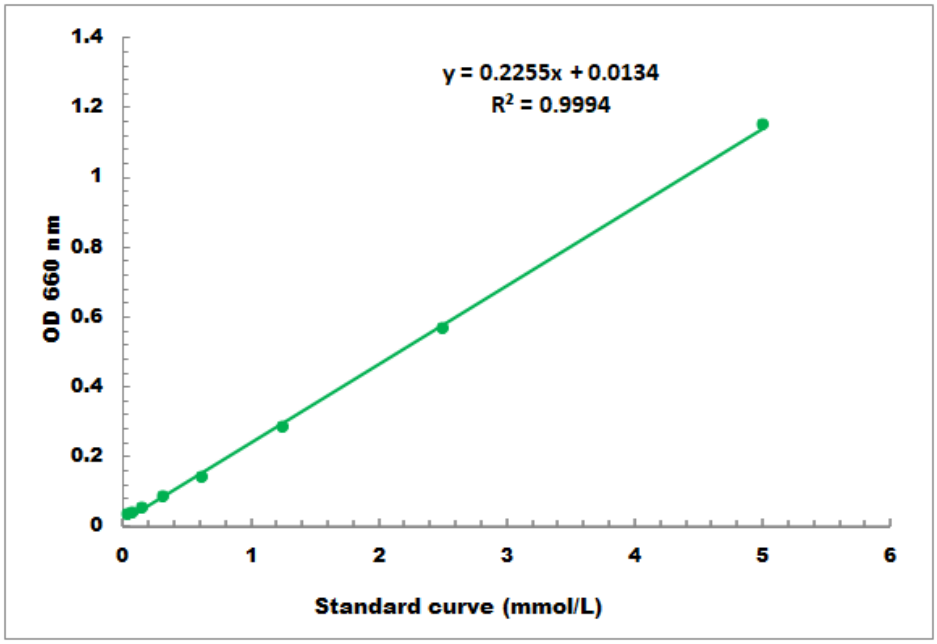
V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

V: the volume of sample in sample preparation, ml

VIII. 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