Glycogen Synthase Activity Colorimetric Microplate Assay Kit

Cat #: orb707344 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Intend Use: Detection and Quantification of Glycogen Synthase (GCS) Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

Detection Range: 4μmol/L - 400 μmol/L

Sample Types: Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids

Assay Principle

Glycogen synthase (EC 2.4.1.11) is a key enzyme in glycogenesis, the process by which glucose is converted into glycogen. It is a glycosyltransferase that catalyzes the reaction between UDP-glucose and (1,4-α-D-glucosyl)n, producing UDP and (1,4-α-D-glucosyl)$n + 1$. In essence, glycogen synthase sequentially adds excess glucose residues to form a polymeric chain for storage as glycogen. Glycogen synthase concentrations are highest in the bloodstream 30 to 60 minutes after intense exercise.

The Glycogen Synthase Activity Colorimetric Microplate Assay Kit provides a sensitive method for measuring glycogen synthase activity in a variety of sample types. Enzyme activity is determined by monitoring the rate of NADH decomposition, with reaction products quantified using a colorimetric readout at 340 nm.

Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer I	5 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer II	15mlx 1	4 °C
Enzyme	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer I to dissolve before use.

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Enzyme: add 1 ml Reaction Buffer II to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 μmol/L.

Materials Required but Not Supplied

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10,000g 4°C for 15 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 10,000g 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.

Assay Procedure

Warm all reagents to 37 °C before use.
Add the following reagents to the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer I	40 μl	--	--
Substrate	10 μl	--	--
Sample	10 μl	--	--
Mix, incubate at 37 °C for 5 mins.			
Standard	--	200 μl	--
Distilled water	--	--	200 μl
Reaction Buffer II	130 μl	--	--

Enzyme	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10thsecond and 70th second.			

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, cannot be mixed and added together.

Calculation of Results

Unit Definition: One Unit of Glycogen Synthase activity is defined as the enzyme reduces 1 µmol of NADH per minute.

1. According to the protein concentration of sample

$GCS\ (U/mg) = (CS_{tandard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$

$= 1.6 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

$GCS\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$

$= 1.6 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / W$

3.According to the quantity of cells or bacteria

$GCS\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$

$= 1.6 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / N$

4. According to the volume of sample

$GCS\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$

$= 1.6 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / (OD_{Standard} - OD_{Blank})$

$C_{Standard}$: the standard concentration, 400 µmol/L = 0.4µmol/ml

$V_{Standard}$: the volume of standard,200 µl = 0.2 ml

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

W: the weight of sample, g

N: the quantity of cellor bacteria, N $\times 10^4$

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

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

T: the reaction time in step 1, 5 minutes.

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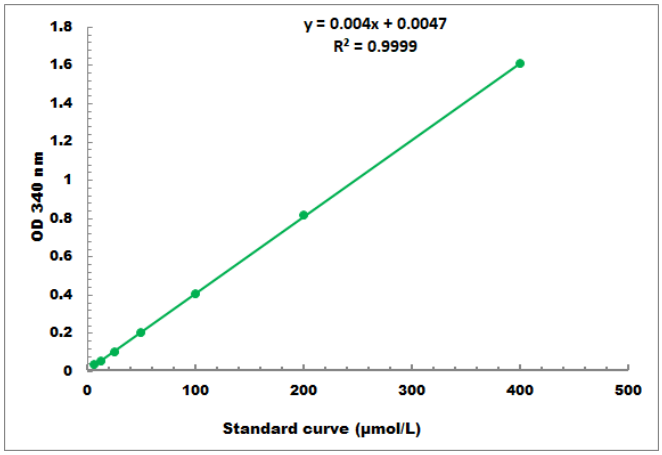
Typical Data

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



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