Glycogen Synthase Assay Kit

Catalog No.: abx298978

Size: 100 Assays

Storage: Store all kit components in the dark. Store Substrate, Enzyme, and Standard at -20°C. Store all other components at 4°C.

Application: For quantitative detection of Glycogen Synthase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Detection Range: 4 µmol/L – 400 µmol/L

Introduction

Glycogen Synthase (GCS) is a key enzyme driving glycogenesis, the anabolic process by which the monosaccharide glucose is converted into the storage polysaccharide glycogen. Specifically, it is a glycosyltransferase enzyme that catalyzes the joining of UDP-glucose to (1,4-α-D-glucosyl)$_n$ chains, which are produced by the action of the enzyme glycogenin. Defects in the activity of Glycogen Synthase result in a reduced ability to store glucose, which can cause symptomatic hypoglycemia.

Glycogen Synthase (GCS) activity results in the decomposition of NADH. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 340 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer A: 5 ml
4. Reaction Buffer B: 15 ml
5. Substrate: 1 vial
6. Enzyme: 1 vial
7. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator
10. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 1 ml of Reaction Buffer A into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Enzyme Solution**
  Add 1 ml of Reaction Buffer B to the Enzyme vial and mix thoroughly to prepare the Enzyme Solution. Ensure that the Enzyme has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of Distilled water to the Standard vial and mix thoroughly. Add 0.2 ml of this solution to 0.8 ml of Distilled water to prepare 1 ml of Standard Solution with a concentration of 400 µmol/L.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 15 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer, then centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Serum, Plasma, and other biological fluids**
  Serum, plasma, and other liquid samples can be analyzed directly.

B. Assay Procedure

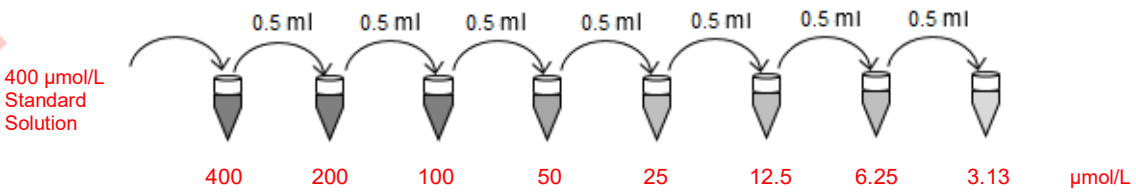
Warm all reagents to 37°C prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 400 µmol/L, 200 µmol/L, 100 µmol/L, 50 µmol/L, 25 µmol/L, 12.5 µmol/L, 6.25 µmol/L and 3.13 µmol/L. Aliquot 0.5 ml of Distilled water into each tube, except the 400 µmol/L tube. The 400 µmol/L Standard Solution will serve as the high standard. Transfer the 0.5 ml of Standard Solution into the 2nd tube, and mix thoroughly (200 µmol/L). Transfer 0.5 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.

   For the blank (0 µmol/L Standard), use pure Distilled water.

   Note: Do not perform the serial dilution directly in the plate wells.



2. Set the sample, standard, control and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 40 µl Reaction Buffer A to the sample wells.
4. Add 10 µl of Substrate Solution to the sample wells.
5. Add 10 µl of sample to the sample wells.
6. Tap the plate gently to mix, and then incubate at 37°C for 5 minutes.

7. Add 200 µl of each Standard Solution to the corresponding standard well.
8. Add 200 µl Distilled water to the blank well.
9. Add 130 µl of Reaction Buffer B to the sample wells.
10. Add 10 µl of Enzyme Solution to the sample wells. Be ready to record the absorbance of the wells immediately after adding the Enzyme Solution. Tap the plate gently to mix.
11. Record the absorbance at 340 nm, at the 10th second and 70th second after adding the Enzyme Solution and mixing.

C. Calculations

One unit of Glycogen Synthase (GCS) activity is defined as the amount of enzyme required to reduce 1 nmol of NADH per minute.

Glycogen Synthase activity per mg of protein:

$$\mathbf{GCS\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times (OD_{Sample(10s)} - OD_{Sample(70s)})}}{\mathbf{V_{Sample} \times C_{Protein} \times (OD_{Standard} - OD_{Blank}) \times T}} = \mathbf{1600} \times \frac{\mathbf{OD_{Sample(10s)} - OD_{Sample(70s)}}}{\mathbf{C_{Protein} \times (OD_{Standard} - OD_{Blank})}}$$

Glycogen Synthase activity per g of sample:

$$\mathbf{GCS\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay} \times (OD_{Sample(10s)} - OD_{Sample(70s)})}}{\mathbf{V_{Sample} \times W \times (OD_{Standard} - OD_{Blank}) \times T}} = \mathbf{1600} \times \frac{\mathbf{OD_{Sample(10s)} - OD_{Sample(70s)}}}{\mathbf{W \times (OD_{Standard} - OD_{Blank})}}$$

Glycogen Synthase activity per 10^4 cells or bacteria:

$$\mathbf{GCS\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay} \times (OD_{Sample(10s)} - OD_{Sample(70s)})}}{\mathbf{V_{Sample} \times N \times (OD_{Standard} - OD_{Blank}) \times T}} = \mathbf{1600} \times \frac{\mathbf{OD_{Sample(10s)} - OD_{Sample(70s)}}}{\mathbf{N \times (OD_{Standard} - OD_{Blank})}}$$

Glycogen Synthase activity per ml of sample:

$$\mathbf{GCS\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times (OD_{Sample(10s)} - OD_{Sample(70s)})}}{\mathbf{V_{Sample} \times (OD_{Standard} - OD_{Blank}) \times T}} = \mathbf{1600} \times \frac{\mathbf{OD_{Sample(10s)} - OD_{Sample(70s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (400 µmol/L = 400 nmol/ml)
$\mathbf{T}$	Reaction time (5 minutes)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of the Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (200 µl = 0.2 ml)