Glycogen Synthase (GCS) Assay Kit

Catalog No.: abx295220

Size: 96 tests

Detection Range: 1.27 U/L – 214.46 U/L

Sensitivity: 1.27 U/L

Storage: Store all components at -20°C in the dark.

Application: For the detection and quantification of GCS activity in tissue homogenates and cell lysates.

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Principle of the Assay

Abbexa's Glycogen Synthase (GCS) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating GCS activity. GCS catalyzes the incorporation of glucose from UDP-glucose into glycogen through α-1,4 glycosidic bond formation. The enzymatic reaction is coupled to NADH consumption, with an absorbance maximum at 340 nm. The decrease in absorbance at 340 nm is directly proportional to GCS activity, which can then be calculated.

Kit components

1. 96-well UV-microplate
2. Substrate: 5 ml
3. Assay Buffer: 28 ml
4. Accelerant Reagent: 4 vials
5. Cofactor Reagent: 4 vials
6. Enzyme Reagent: 0.2 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (340 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Centrifuge and centrifuge tubes
7. Sonicator
8. Mechanical homogenizer
9. Vortex mixer
10. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Accelerant Working Solution:** Reconstitute 1 vial of Accelerant Reagent with 2 ml of Assay Buffer. Mix thoroughly until fully dissolved. Unused Accelerant Working Solution can be stored at -20°C for up to 2 days in the dark.
- **Cofactor Working Solution:** Reconstitute 1 vial of Cofactor Reagent with 6 ml of double-distilled water. Mix thoroughly until fully dissolved. Cofactor Working Solution should be prepared immediately before use.
- **Enzyme Working Solution:** Prepare enough Enzyme Working Solution for the wells to be tested. Mix Enzyme Reagent with Assay Buffer in a ratio of 1 : 24. For example, prepare 1000 µl of Enzyme Working Solution by mixing 40 µl of Enzyme Reagent with 960 µl of Assay Buffer. Unused Enzyme Working Solution can be stored at -20°C for up to 3 days in the dark.
- **Reaction Working Solution:** Prepare enough Reaction Working Solution for the wells to be tested. Mix Substrate, Enzyme Working Solution, Cofactor Working Solution, Accelerant Working Solution and Assay Buffer in a ratio of 4 : 4 : 2 : 2: 5. For example, prepare 170 µl of Reaction Working Solution by mixing 40 µl of Substrate, 40 µl of Enzyme Working Solution, 20 µl of Cofactor Working Solution, 20 µl of Accelerant Working Solution and 50 µl of Assay Buffer. Mix thoroughly before use and avoid generating bubbles during preparation and dispensing. The Reaction Working Solution is stable for up to 4 hours in the dark.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of cold normal saline (0.9% NaCl). Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 12,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately.
- **Cell lysates:** Collect at least 1×10^6 cells for analysis. Wash the cells with cold PBS (0.01 M, pH 7.4). Per 1×10^6 cells, add 200 µl of cold normal saline (0.9% NaCl). Sonicate at 4 °C for homogenization. Centrifuge at 12,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately.

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Note: To calculate GCS activity in tissue homogenates and cell lysates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097193)

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Mouse Liver Tissue Homogenate	2 – 3
10% Mouse Muscle Tissue Homogenate	5 – 10
1 × 10^6 HL-60 Cells	1
1 × 10^6 K562 Cells	1
1 × 10^6 A549 Cells	1
1 × 10^6 RAW264.7 Cells	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the performance of this kit. It is recommended to use mechanical lysis methods only for tissue homogenates and cell lysates.

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B. Assay Procedure

1. Mark positions on the 96-well microplate for each blank and sample. *It is strongly recommended to test all wells in duplicate*.
2. Add 20 µl of normal saline (0.9% NaCl) to the blank wells.
3. Add 20 µl of sample to the sample wells. *For optimal assay performance, the number of sample wells analyzed in a single run should not exceed 10.*
4. Add 170 µl of Reaction Working Solution to all wells.
5. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for approximately 5 seconds.
6. Measure the OD of each well with a microplate reader at 340 nm and record the value as A_1.
7. Cover with a plate sealer, then incubate at 25°C for 10 minutes.
8. Unseal the plate, then measure the OD of each well with a microplate reader at 340 nm and record the value as A_2.

   Note: The A_2 value should be greater than 0.400. If the A_2 value is below 0.400, dilute the sample appropriately and repeat the assay.

C. Calculation of Results

For each sample, calculate the difference in absorbance between the two measurements: $\Delta OD = A_1 - A_2$.

Note: If ΔOD is less than 0.005, the incubation time may be extended appropriately. The activity calculation should be adjusted according to the actual incubation time used.

$$\text{GCS activity (U/g)} = \frac{(\Delta OD_{Sample} - \Delta OD_{Blank}) \times V_{Total}}{\varepsilon \times d \times T \times V_{Sample} \times C_{Protein}} \times F \times 10^6$$

$$= \frac{(\Delta OD_{Sample} - \Delta OD_{Blank})}{C_{Protein}} \times F \times 254.56$$

where:

ΔOD_{Sample}	$A_1 - A_2$ value of the sample.
ΔOD_{Blank}	$A_1 - A_2$ value of the blank
V_{Sample}	Volume of sample added to the microplate (0.02 ml)
V_{Total}	Volume of total reaction (0.19 ml)
$C_{Protein}$	Concentration of protein in sample (g/L)
ε	Molar extinction coefficient of NADH at 340 nm, (6.22 × 10^3 L/mol/cm)
d	Optical path length (0.6 cm)
T	Time for the reaction (10 minutes)
10^6	Conversion factor (1 mol = 1 × 10^6 μmol)
F	Dilution factor of the sample

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Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.