（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1154-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：1.27-214.46 U/L

Elabscience® Glycogen Synthase(GCS) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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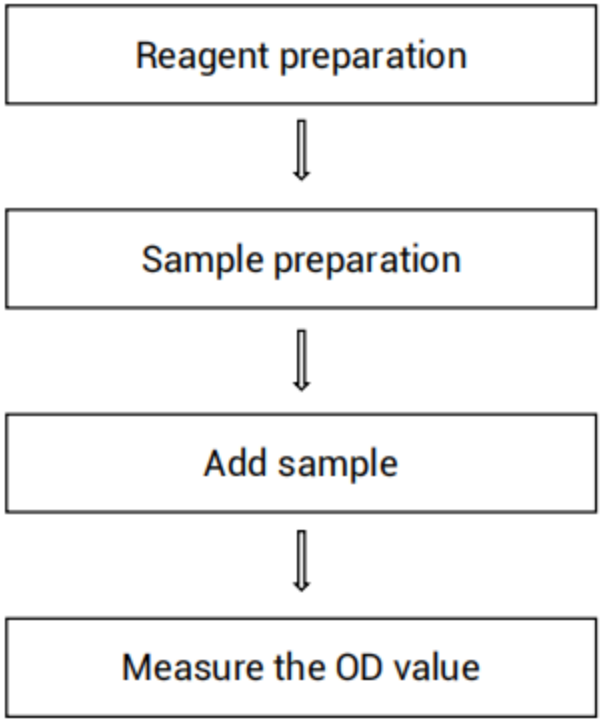
Assay summary



Intended use

The kit is suitable for detecting the activity of Glycogen Synthase (GCS) in animal tissues and cells.

Detection principle

Glycogen synthase (GCS) adds the sugar group of UDPG to the non-reducing end of the existing glycogen or glycogen protein, linking them through an α-1,4 glycosidic bond. GCS is the rate-limiting enzyme in the glycogen synthesis process of the animal body and also the main target enzyme for insulin action. It plays an important role in sugar metabolism and maintaining relatively stable blood sugar levels.

GCS catalyzes substrate reactions to consume NADH. Measuring the decline rate of NADH at 340 nm can reflect the activity of GCS.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	-20°C, 6 months, shading light
Reagent 2	Accelerant	powder × 2 vials	powder × 4 vials	-20°C, 6 months, shading light
Reagent 3	Co-factor	powder × 2 vials	powder × 4 vials	-20°C, 6 months, shading light
Reagent 4	Substrate	2.5 mL × 1 vial	5 mL × 1 vial	-20°C, 6 months, shading light
Reagent 5	Enzyme Reagent	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 6 months, shading light
	UV-Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (340 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Accelerant Working Solution preparation:

Dissolve one vial of accelerant with 2 mL of buffer solution, mix well to dissolve. Keep it on ice and protected from light during use. The accelerant working solution can be aliquoted and stored at -20°C for 2 days protected from light.

③ Co-factor Working Solution preparation:

Dissolve one vial of co-factor with 6 mL of double distilled water, mix well to dissolve. Keep it on ice and protected from light during use. The co-factor working solution can be freshly prepared before use. Stable for 4 h when stored at -20°C protected from light.

④ Enzyme Reagent Working Solution preparation：

Before testing, please prepare sufficient according to the test wells. For example, prepare 1000 μL of enzyme reagent working solution (mix well 960 μL of buffer solution and 40 μL of enzyme reagent). The enzyme reagent working solution should be prepared on spot protected from light and used up within 3 days.

⑤ Reaction Working Solution preparation：

For each well, prepare 170 μL of reaction working solution (mix well 50 μL of buffer solution, 20 μL of accelerant working solution, 20 μL of co-factor working solution, 40 μL of substrate and 40 μL of enzyme reagent working solution). Stable for 4 h protected from light.

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
② Wash tissue in cold PBS (0.01 M, pH 7.4).
③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.
④ Centrifuge at 12000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection. The prepared sample supernatant store at -20°C and use within 4 h.
⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
② Wash cells with PBS (0.01 M, pH 7.4).
③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.
④ Centrifuge at 12000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection. The prepared sample supernatant store at -20°C and use within 4 h.
⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenization	2-3
10% Mouse muscle tissue homogenization	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: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The Reaction Working Solution should be thoroughly mixed. Avoid generating bubbles when adding.

② Try to use fresh samples for the measurement. The sample should be measured within 4 hours after processing.

③ The number of detection wells in each experiment should be controlled to be within 10.

④ If the measured value ΔA of the sample is less than 0.005, the measurement time can be appropriately extended. The corresponding calculation formula needs to be modified by yourself.

⑤ The value of detection well A_2 should be greater than 0.4. If it is lower than 0.4, the sample needs to be diluted.

Operating steps

① Blank well: Add 20 μL of normal saline (0.9% NaCl) to the corresponding wells. (just need to conduct 1-2 tests).
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 170 μL of Reaction Working Solution to each well.

③ Mix fully with microplate reader for 5 s. Measure the initial OD value (A_1) of each well at 340 nm with microplate reader.

④ Incubate at 25°C for 10 min. Measure the OD value (A_2) of each well. (The standard curve is fitted to the standard well in A_2 value).

Calculation

The sample:

Tissue or cell sample:

Definition: One unit of enzyme is defined as the amount of 1 g protein will consume 1 μmol of NADH per 1 min at 25°C.

$$\text{GCS activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \times \frac{V_1}{V_2} \times 10\text{^}6 \div T \div C_{pr} \times f$$

$$= (\Delta A_{sample} - \Delta A_{blank}) \times 254.56 \div C_{pr} \times f$$

[Note]

ΔA_{sample}: The OD value of the sample well changes, $A_{sample1} - A_{sample2}$.

ΔA_{blank}: The OD value of the sample well changes, $A_{blank1} - A_{blank2}$.

ε: The molar extinction coefficient of at 340 nm,6.22 × 10^3 L/mol/cm.

d: Optical path, 0.6 cm.

V_1: The volume of reaction system, 0.19 mL.

V_2: The volume of sample added to the reaction system, 0.02 mL.

10^6: 1 mol = 1×10^6 μmol.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: Reaction time, 10 min.

254.56:calculated value.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rats serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	20	40	80
%CV	4.2	4.3	3.5

Inter-assay Precision

Three rats serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	20	40	80
%CV	9.7	5.8	7.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	20	40	80
Observed Conc. (U/L)	0.145	0.222	0.297
Recovery rate (%)	106	100	100

Sensitivity

The analytical sensitivity of the assay is 1.27 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

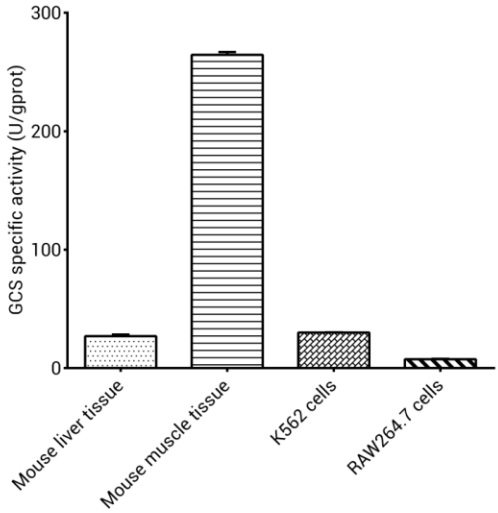
Example analysis：

Take 20 μL of the 2-fold diluted 10% mouse liver tissue homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

The A_1 of blank is 1.731, the A_2 of blank is 1.715, ΔA_{blank} = 1.731 – 1.715 = 0.016. The A_1 of sample is 1.685, the A_2 of sample is 1.102, ΔA_{sample} = 1.685 – 1.102 = 0.583, the concentration of protein is 10.43 gprot/L, and the calculation result is:

GCS activity(U/gprot) = (0.583 - 0.016) × 254.56 ÷ 10.434 × 2 = 27.66 U/gprot

ect 10% mouse liver tissue homogenization (the concentration of protein is 10.43 gprot/L, dilute for 2 times), 10% mouse muscle tissue homogenization (the concentration of protein is 4.79 gprot/L, dilute for 10 times), 1×10^6 K562 cells (the concentration of protein is 1.96 gprot/L) and 1×10^6 RAW264.7 cells (the concentration of protein is 0.80 gprot/L), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.