PRODUCT INFORMATION & MANUAL

Glycogen Assay Kit (Fluorometric)

NBP3-24502

For research use only.
Not for diagnostic or therapeutic procedures.

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Glycogen Assay Kit (Fluorometric)

Catalog No: NBP3-24502

Method: Fluorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.06 μg/mL

Detection range: 0.06-4.0 μg/mL

Average intra-assay CV (%): 3.4

Average inter-assay CV (%): 6.6

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used for determination of glycogen content in animal liver and muscle tissue samples.

▲Background

Glycogen is mainly produced by liver and muscle. Glycogen content is mainly regulated by glycogen synthase and glycogen phosphorylase. In the liver, glycogen acts as a glucose store for other tissues and maintains blood glucose levels. In muscle, glycogen is mainly used as energy for the supply of adenosine triphosphate (ATP) during muscle contraction. Due to the lack of glucose-6-phosphatase, muscle glycogen cannot maintain the level blood glucose.

▲Detection principle

Glycogen produces glucose under the action of starch glycosidase, and glucose is catalyzed by glucose oxidase to produce hydrogen peroxide. In the presence of the peroxidase, hydrogen peroxide be oxidized to produce the fluorescence substrate. The fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 587 nm is proportional to the glycogen content.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution A	30 mL × 1 vial	-20℃ , 12 months
Reagent 2	Buffer Solution B	8 mL × 1 vial	-20℃ , 12 months
Reagent 3	Probe	0.24 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent A	Powder × 1 vial	-20℃ , 12 months
Reagent 5	Enzyme Reagent B	Powder ×1 vial	-20℃ , 12 months
Reagent 6	0.1 mg/mL Glucogen Standard Solution	0.5 mL × 1 vial	-20℃ , 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Incubator, Vortex mixer, Water bath, Centrifuge

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. After preparation of the reaction working solution, it must be stored with shading light.
2. Prevent the formulation of bubbles when the reagents is added into the microplate.
3. Since the tissues continue to have a relatively high rate of anaerobic metabolism after death, the glucose content in the tissues decreases rapidly to undetectable levels, resulting in further hydrolysis of glycogen and a significant decrease of the content. To accurately measure tissue glycogen, if it is not possible to measure it immediately, effective inactivation means should be adopted after the sample is taken out. The tissue can be immediately moved to liquid nitrogen, and then ground in liquid nitrogen and stored at -20 or -80℃ .

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of reagent 4 working solution:

   Dissolve reagent 4 with 1.2 mL of reagent 1. Prepare the needed amount before use. The prepared solution can be aliquoted and stored at -20℃ for 1 week.
3. The preparation of reagent 5 working solution:

   Dissolve reagent 5 with 0.24 mL of reagent 2. Prepare the needed amount before use. The prepared solution can be aliquoted and stored at -20℃ for 1 week.
4. The preparation of reaction working solution:

   Mix the reagent 2, reagent 3 and reagent 5 working solution at a ratio of 46:2:2 fully. Prepare the fresh solution before use and store it with shading light.
5. The preparation of 25 μg/mL glucogen standard:

   Dilute 100 μL of 0.1 mg/mL glucogen standard solution with 300 μL of reagent 1 and mix fully.

▲Sample preparation

1. Animal tissue sample:

   Take 0.1g fresh tissue, add 0.9 mL of double distilled water according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath and incubate at 95℃ for 10 min. Cool with ice water and centrifuge at 12000 g for 10 min at 4℃ , then take the supernatant for detection.
2. Cell sample:

   Add double distilled water at a ratio of cell number (10^6): homogenization medium (μL) =1: 200. Sonicate or mechanical homogenate and incubate at 95℃ for 10 min. Cool with ice water and centrifuge at 12000 g at 4℃ for 10 min, then take the supernatant and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.06-4.0 μg/mL).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	3000-5000
10% Mouse muscle tissue homogenate	10-20

Note: The diluent is reagent 1. Liver samples can be diluted step by step.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

[Note]: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 25 μg/mL standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 μg/mL. Reference is as follows:

Number	Standard concentrations (μg/mL)	25 μg/mL standard (μL)	Reagent 1 (μL)
A	0	0	500
B	0.5	10	490
C	1.0	20	480
D	1.5	30	470
E	2.0	40	460
F	2.5	50	450
G	3.0	60	440
H	4.0	80	420

The measurement of samples

1) Standard well: add 50 μL of standard with different concentrations into the well.
   Sample well: add 50 μL of sample into the well.
   Control well: add 50 μL of sample into the well.
2) Add 20 μL of reagent 4 working solution to standard well and sample well. Add 20 μL of reagent 1 to control well.
3) Add 50 μL of reagent reaction working solution to each well.
4) Mix fully with microplate reader for 5 s and stand at room temperature for 30 min with shading light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. The fluorescence intensity of sample well recorded as F_1, and the fluorescence intensity of control well recorded as F_2.

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	50		
Sample (μL)		50	50
Reagent 4 working solution (μL)	20	20	
Reagent 1 (μL)			20
Reaction working solution (μL)	50	50	50
Mix fully and stand at room temperature for 30 min with shading light. Measure the fluorescence intensity. The fluorescence intensity of sample well recorded as F_1, and the fluorescence intensity of control well recorded as F_2.			

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: y= ax + b.

1. Cell sample:

Glycogen content (μg/10^6 cells) = (ΔF- b) ÷ a × f ÷ (n÷V_1)

2. Tissue sample:

Glycogen content (μg/mg wet weight) = (ΔF- b) ÷ a × f ÷ (m÷V_2)

Note:

y: $F_{Standard} - F_{Blank}$. (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: Change of fluorescence intensity of sample ($F_1 - F_2$) - F_{Blank}.

f: Dilution factor of sample before tested.

m: The weight of tissue sample, 100 mg.

n: The number of cells. For example, the number of cells is $5*10^6$, n is 5.

V_1: The volume of double distilled water added during the preparation of cells, mL.

V_2: The volume of double distilled water added during the preparation of tissue, mL.

Appendix I Data

▲Example analysis

For mouse liver tissue, take 50 μL of tissue supernatant diluted for 4000 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 2283 x + 106.49, tthe average fluorescence value of the sample (F_1) is 3961, the average fluorescence value of the control (F_2) is 1103, the average OD value of the blank is1444, ΔF = 3961 – 1103 – 1444 = 1414, and the calculation result is:

Glycogen content (μg/mg wet weight)=(1414-106.49)÷2283×4000÷(100÷0.9)

=20.62 μg/mg wet weight