PRODUCT INFORMATION & MANUAL

Glycerol Assay Kit (Colorimetric)

NBP3-25812

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

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Glycerol Assay Kit (Colorimetric)

Catalog No: NBP3-25812

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.01mmol/L

Detection range: 0.01-1.00 mmol/L

Average intra-assay CV (%): 4

Average inter-assay CV (%): 8

- ▲ 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 measure glycerol in serum (plasma), animal tissue and cell samples.

▲Detection principle

Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, which is further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator of triglyceride hydrolysis reaction and detection is more convenient.

Glycerol is transformed by enzyme to produce hydrogen peroxide, which is catalyzed by oxidase in the presence of 4-amino-antipyrprid and phenol to produce red quinones, the color of which is proportional to the content of glycerol.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Working Solution	25 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 2	1.0 mmol/L Standard	1 mL × 2 vials	2-8℃ , 12 months, shading light
	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

Water bath, Microplate reader (500-520 nm, optimum wavelength: 510 nm).

Reagents:

Double distilled water, Isopropanol (AR), PBS(0.01 M, pH 7.4).

⚠ 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. Touch the bottom of the plate when adding standards and samples.
2. There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲ Reagent preparation

Bring all reagents to room temperature before use.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add isopropanol at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then inactivate at 70°C water bath for 10 min, centrifuge at 10000 g for 10 min, take the supernatant for detection.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6) : isopropanol (μL) = 1 : 100. Sonicate or mechanical homogenate in ice water bath. Then inactivate at 70°C water bath for 10 min, centrifuge at 10000 g for 10 min, take the supernatant 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 (0.01-1.00 mmol/L).

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2
10% Mouse kidney tissue homogenate	1
10% Mouse spleen tissue homogenate	1
Cell	1

Note: The diluent is double distilled water.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.1	20	180
C	0.2	40	160
D	0.4	80	120
E	0.5	100	100
F	0.6	120	80
G	0.8	160	40
H	1.0	200	0

2. The measurement of samples

(1) Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

(2) Add 250 μL of reagent 1 to each well.

(3) Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min. Measure the OD value of sample well at 510 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	10	
Sample (μL)		10
Regent 1 (μL)	250	250
Mix fully and incubate at 37℃ for 10 min. Measure the OD value of sample well.		

▲Calculation

Plot the standard curve by using OD value 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 OD value of sample. The standard curve is: y= ax + b.

1. Serum/plasma sample:

$$\text{Glycerol content (mmol/L)} = (\Delta A_{510} - b) \div a \times f$$

2. Tissue sample:

$$\text{Glycerol content (mmol/kg wet weight)} = (\Delta A_{510} - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\text{Glycerol content (μmol/10^6)} = (\Delta A_{510} - b) \div a \times V \div N \times f$$

Note:

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

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{510}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

V: The volume of isopropanol, mL.

m: The weight of sample, g.

N: The number of cells/10^6 (For example, the number of cells is 5×10^6, N is 5).

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For mouse kidney tissue, take 10 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.536 x – 0.003, the OD value of the blank is 0.072, the OD value of the sample is 0.244, and the calculation result is:

Glycerol content (mmol/kg wet weight) = (0.244 - 0.072 + 0.003) ÷ 0.536 × 0.9 ÷ 0.1

= 2.93 mmol/kg wet weight