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CheKine™ Micro Glycerol Content Assay Kit

Cat #: KTB2201 Size: 48 T/48 S 96 T/96 S

	Micro Glycerol Content Assay Kit		
REF	**Cat #:** KTB2201	LOT	**Lot #:** Refer to product label
	Detection range: 7.81-1,000 nmol/mL		**Sensitivity:** 7.81 nmol/mL
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, and then further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator for the hydrolysis reaction of triglyceride, which is more convenient for detection. CheKine™ Micro Glycerol Content Assay Kit can be used to detect biological samples such as animal and plant tissues, cells, plasma, serum or other liquid samples. In the kit, glycerol is phosphorylated to glycerol 3-phosphate by glycerol kinase in the presence of ATP, and then oxidized by glycerol phosphate oxidase to produce hydrogen peroxide. Under the action of catalase, the color substrate is converted to benzoquinimide, and there is an absorption peak at 505 nm. The content of glycerol can be calculated by measuring the absorbance of this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	15 mL	30 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS, isopropyl alcohol

• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Working Reagent I: Prepared before use. Add 7 mL Reagent III for 48 T and 14 mL Reagent III for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Reagent II: Prepared before use. Add 7 mL Reagent III for 48 T and 14 mL Reagent III for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 month.

Working Reagent: Prepared before use. Mix Working Reagent I and Working Reagent II in a ratio of 1: 1. Working Reagent is freshly prepared.

Reagent III: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Standard: Ready to use as supplied; 1 μmol/mL glycerol Standard; Equilibrate to room temperature before use; Store at 4℃, protected from light. Using 1 μmol/mL glycerol Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	100 μL of 1 μmol/mL Standard	0	1,000
Std.2	50 μL of Std.1 (1,000 nmol/mL)	50	500
Std.3	50 μL of Std.2 (500 nmol/mL)	50	250
Std.4	50 μL of Std.3 (250 nmol/mL)	50	125
Std.5	50 μL of Std.4 (125 nmol/mL)	50	62.5
Std.6	50 μL of Std.5 (62.5 nmol/mL)	50	31.25
Std.7	50 μL of Std.6 (31.25 nmol/mL)	50	15.63
Std.8	50 μL of Std.7 (15.63 nmol/mL)	50	7.81
Blank	0	50	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal tissues: Weigh 0.1 g tissue, rinse the tissue with PBS, blot with filter paper, add 1 mL isopropyl alcohol and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL isopropyl alcohol to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample. Because isopropyl alcohol can cause protein degeneration, the sample needs to be extracted with deionized water according to the steps separately.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Control Well (μL)	Test Well (μL)
Sample	0	0	0	5
Isopropyl Alcohol	0	0	5	0
Standard	0	5	0	0
Deionized Water	5	0	0	0
Working Reagent	200	200	200	200

3. Mix well, immediately detect optical density at 505 nm as A_1. Incubate for 10 min at 37°C. Measure optical density of 10 min at 505 nm again as A_2, $\Delta A= A_2-A_1$. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$.

Note: The Control Well, the Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $\Delta\Delta A_{Test}$ is less than 0.004, increase the sample quantity appropriately. If $\Delta\Delta A_{Test}$ is greater than 1,000 nmol/mL of $\Delta\Delta A_{Standard}$, the sample can be appropriately diluted with isopropyl alcohol, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of $\Delta\Delta A_{Test}$ is brought into the equation to get x (nmol/mL).

2. Calculation of the glycerol content

(1) Calculated by protein concentration

Glycerol (nmol/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)$**=x÷Cpr**

(2) Calculated by fresh weight of samples

Glycerol (nmol/mg fresh weight)=$(V_{Sample}\times x)\div(w\times V_{Sample}\div V_{Total\ sample})$**=x÷w**

(3) Calculated by number of cells

Glycerol (nmol/10^6 cell)=$(V_{Sample}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})$**=x÷n**

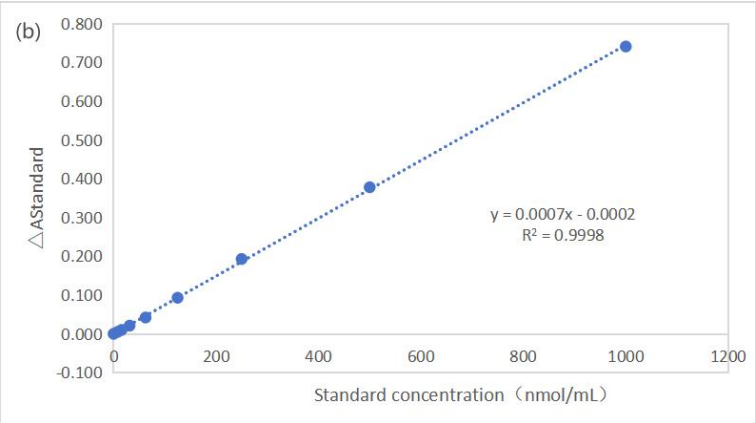
(4) Calculated by volume of liquid samples

Glycerol (nmol/mL)=$(V_{Sample}\times x)\div V_{Sample}$**=x**

V_{Sample}: Added the sample volume, 0.005 mL; $V_{Total\ sample}$: Added the isopropyl alcohol volume, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; n: Number of cells, calculated in units of one million.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.



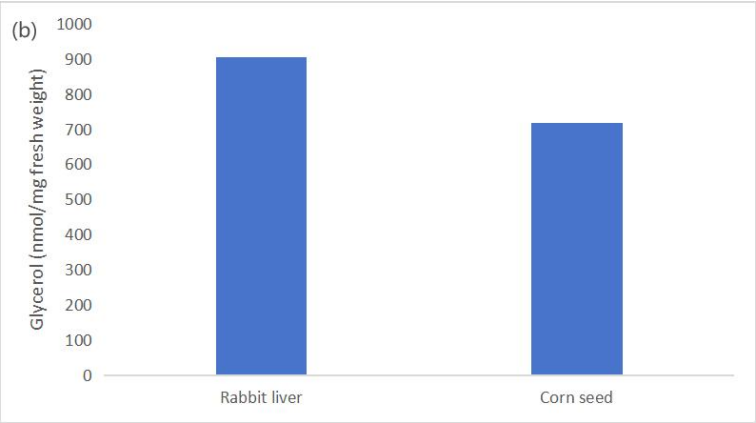


Figure 1. (a) Standard curve of glycerol. (b) Determination of glycerol content in rabbit liver and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.