CheKine™ Glycogen Assay Kit (Colorimetric)

Cat #: KTB1340 Size: 48 T/96 T

	Glycogen Assay Kit (Colorimetric)		
REF	**Cat #:** KTB1340	LOT	**Lot #:** Refer to product label
	Detection range: 0.003125-0.25 mg/mL		**Sensitivity:** 0.003125 mg/mL
	Applicable samples: Animal tissues, Bacteria, Cells		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Glycogen is a polymer polysaccharide composed of glucose. It is one of the main storage forms of glucose. It is mainly stored in liver and muscle as reserve energy, which is called liver glycogen and muscle glycogen, respectively. Liver glycogen can regulate the concentration of blood glucose. When blood glucose rises, glycogen can be synthesized in the liver. When blood glucose decreases, liver glucose principle decomposes into glucose to supplement blood glucose. Therefore, liver glycogen is very important to maintain the relative balance of blood glucose. Muscle glycogen is the storage form of glucose in muscle. When strenuous exercise consumes a large amount of blood glucose, muscle glycogen cannot be decomposed directly into blood glucose. It was decomposed to produce lactic acid and circulate to the liver with blood. It is transformed into liver glycogen and glucose by gluconeogenesis. CheKine™ Glycogen Assay kit can detect glycogen concentration in animal tissues, bacteria and cells. The principle of kit is strong alkaline extract glycogen. under strong acidic conditions, blue compounds with anthrone chromogenic agent with characteristic absorption peak at 620 nm. In a certain concentration range, glycogen concentration is linearly related to 620 nm absorbance. According to the standard curve, the glycogen concentration in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Chromogen	1	1	4℃, protected from light
Standard	1 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 620 nm
- Refrigerated Centrifuge, Water bath
- 96-well microplate with clear flat bottom, Precision Pipettes, Disposable Pipette Tips

• Deionized Water, Concentrated Sulfuric Acid

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Chromogen: Add 6 mL deionized water to dissolve the powder, then slowly pour into 24 mL concentrated sulfuric acid, fully dissolve and mix. 4℃ avoid light for one week.
Standard: 1 mg/mL. Store at 4℃.
Standard curve setting: Dilute 1 mg/mL standard with deionized water to 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 mg/mL standard solution as shown in the table below.

	Volume of Standard	Deionized Water (μL)	Concentration (mg/mL)
Std.1	50 μL 1 mg/mL	150	0.25
Std.2	160 μL of Std.1	240	0.1
Std.3	200 μL of Std.2	200	0.05
Std.4	200 μL of Std.3	200	0.025
Std.5	200 μL of Std.4	200	0.0125
Std.6	200 μL of Std.5	200	0.00625
Std.7	200 μL of Std.6	200	0.003125

Sample Preparation

1. Tissue samples: Weigh 0.1 g tissue, put it in 10 mL test tube, add 0.75 mL Extraction Buffer, boil 20 min (cover tightly to prevent water loss). shake test tube every 5 min, mix well. When the tissue is dissolved, dilute with deionized water to 5 mL, centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.
2. Bacteria or cells: collect 5 million bacteria or cells into EP tubes, wash with PBS, add 0.75 mL Extraction Buffer, Ultrasonic lysis of bacteria or cells (200 W, work 3 s, intermittent 10 s, work 30 times). Transferred to 10 mL tube, boil 20 min (cover tightly to prevent water loss), shake the tube every 5 min, mix well. Dilute with deionized water to 5 mL, mix well, 8,000 g, 25℃ centrifuge 10 min, Use supernatant for assay.

Assay Procedure

1．Preheat the microplate reader for more than 30 min, and adjust the wavelength to 620 nm.
2．Add the following reagents respectively into each EP tube:

	Blank tube (μL)	Standard tube (μL)	Test tube (μL)
Sample	0	0	60
Standard	0	60	0
Deionized Water	60	0	0
Chromogen	240	240	240

3. Mix well, incubate in 95℃ 10 min (Cover tightly to prevent moisture evaporation), add 200 uL of the reaction mix to 96-well plate. Measure absorbance (OD620 nm) for in a microplate reader. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.
Note: Extraction Buffer is corrosive, Chromogen is toxic. Please take protective measures when operating. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

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 y axis and the $\Delta A_{Standard}$ as the x axis, draw the standard curve y=kx+b. Substitute the ΔA_{Test} into the equation to obtain the y value (mg/mL).

2．Calculated by sample quality

Glycogen (mg/g)=1.11×(y×V_{Sample})÷(W×V_{Sample}÷$V_{Total\ sample}$)×n=**5.55×y÷W×n**

3．Calculated by protein concentration

Glycogen (mg/mg prot)=1.11×(y×V_{Sample})÷(V_{Sample}×Cpr)×n=**1.11×y÷Cpr×n**

4．Calculated by the number of bacteria or cells

Glycogen (mg/10^4 cells) =1.11×(y×V_{Sample})÷(the number of bacteria or cells×V_{Sample}÷$V_{Total\ sample}$)×n=**5.55×y÷the number of bacteria or cells×n**

Where: 1.11: 100 μg glucose with anthrone reagent color equivalent to 111 μg glycogen. W: sample mass, g. V_{Sample}: the sample volume to be tested, 0.06 mL. $V_{Total\ Sample}$: sample volume, 5 mL. Cpr: Sample protein concentration, mg/mL. The number of bacteria or cells: 10^4. n: the sample dilution factor.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay:

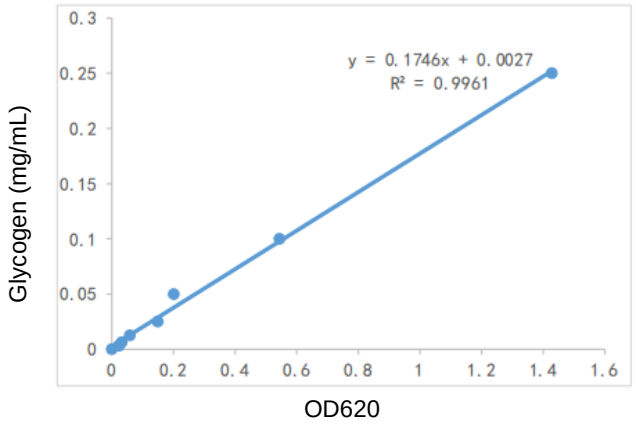

Fig. Standard Curve of Glycogen

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Plant Soluble Sugar Colorimetric Assay Kit (Colorimetric)
KTB1330	CheKine™ Blood Glycogen Colorimetric Assay Kit (Colorimetric)
KTB1350	CheKine™ Total Carbohydrate Colorimetric Assay Kit (Colorimetric)
KTB1360	CheKine™ Reducing Sugar (RS) Colorimetric Assay Kit (Colorimetric)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.