Ver.250501

Glycogen Colorimetric Assay Kit (Liver/Muscle Samples)

E-BC-K073-S(50T/48S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

1. Assay principle

Under the presence of concentrated sulfuric acid, glycogen can be dehydrated to furfural derivatives. Furfural derivatives can form blue compound withanthracenone. The concentration of the compound can be measured bycolorimetric quantification at 620 nm with glucose standard buffer of sametreatment. Glycogen is quite stable in concentrated alkali solution. Heating thetissue sample in concentrated alkali solution before color development willremove other components and keep the glycogen.

2. Reagent composition & preparation

Reagent	Volume	Storage
Alkaline solution	40mL × 1	2-8°C
Glucose standard stock solution (1mg/mL)	1mL × 1 vial	2-8°C
Chromogenic agent	Powder × 6 vials	2-8°C, in dark

- Glucose standard working solution: Mix glucose standard stock solution with distilled water at ratio of 1:99, this working solution should be used soon after preparation, it can be stored for 1 day. Final concentration is 0.01mg/mL

- Chromogenic agent preparation: Add 25mL concentrated sulphuric acid, mix sufficiently, this chromogenic agent should be used soon after preparation

Note:

a) Do not keep open the container with concentrated sulphuric acid for a long tim.

b) Container & volumetric cylinder for chromogenlc agent preparation must be absolute dry or Chromogenic reagent can not be dissolved completely

c) When you prepare chromogenic agent, please pour powder in a flask, add small amount of sulphurlc acid (about 10mL), continuously stir with a glass rod in order to dissolve completely, then make up the volume to 25mL with sulphuric acld, mix thoroughly. This mixture can be stored at room temperature away from light.

3. Operation procedure

1) Sample pretreatment:

a) Sampling: Take fresh liver or muscle sample, rinse using physiological saline, dry on a filter paper, weigh (Use 100mg tissue. Do not use more than 100mg for the assay).

b) Hydrolysis: Sample weight (mg): Alkaline solution(μL)=1:3, add them in test tube, place in boiling water bath, cool to room temperature with flowing water.

Example
If liver sample weight is 75mg, then alkaline solution volume should be 225μL.
If muscle sample weight is 85mg, then alkaline solution volume is 255μL.

Note: Seal test tube by handi-wrap before boilng in order to avoid water evaporation, pierce a small hole on handi-wrap by needle for air thermal expansion.

c) Convert glycogen hydrolysate to glycogen determination solution:

For Liver glycogen determination solution, dilute the liver sample 100 times with distilled water

Volume of distilled water = Sample weight×100- Sample weight×4*

= Sample weight×96

For Muscle glycogen determinatlon solution, dilute the muscle sample 20 times with distilled water

Volume of distilled water = Sample weight× 20- Sample weight × 4*

= Sample weight×16

***Note: Sample is 4 times diluted during hydrolysis (step b).**

Example
Volume of distilled water for liver sample = 75×96=7200μL =7.2mL
Volume of distilled water for muscle sample = 85×16 = 1360μL =1.36mL

2) Operation table:

	Blank (B)	Standard (S)	Sample (T)
Distilledwater(mL)	1.0	-	0.9
Glucose standard working solution (mL)	-	1.0	-
Determinationsolution(mL)	-	-	0.1
Chromogenic agent(mL)	2	2	2

Mix thoroughly, place in boiling water bath for 5minutes,cool to room temperature, transfer in cuvettes of 1cm lightpath, measure OD values of all tubes at 620nm (adjust zero with the blank tube).

OD Blank = A_B

OD Standard = A_S $\Delta A_S = A_S - A_B$

OD Sample = A_T $\Delta A_T = A_T - A_B$

d) Calculation

Glycogen content (mg/tissue) = $\Delta A_T \div \Delta A_S \times Sc \times D \times 10 \div 1.11$

Sc : Standard concentration (0.01mg/mL)
D : Sample dilution (100 for liver; 20 for muscle)
10 : Dilution during assay
1.11 : Coefficient of glucose-glycogen coversion (100µg glycogen reacted with anthrone equals to absorbance of111µg glucose reacted with anthrone)

Example

Liver sample
$\Delta A_S = 0.174$; $\Delta A_T = 0.223$
Liver Glycogen content (mg/g tissue) = $0.223 \div 0.174 \times 0.01 \times 100 \times 10 \div 1.11$
= 11.546 mg/g tissue

Muscle sample
$\Delta A_S = 0.174$; $\Delta A_T = 0.171$
Liver Glycogen content (mg/g tissue) = $0.171 \div 0.174 \times 0.01 \times 20 \times 10 \div 1.11$
= 1.77 mg/g tissue

APPENDIX: Fatty liver sample glycogen assay

1. **Sample pretreatment:**

   a) **Sampling:** Take fresh liver or muscle sample, rinse using physiological saline, dry on a filter paper, weigh (Use 100mg tissue. Do not use more than 100mg for the assay).

   b) **Hydrolysis:** Sample weight (mg): Alkaline solution (μL) = 1:3, add them in test tube, place in boiling water bath, cool in flowing water.

   Note: Seal test tube by handi-wrap before boiling inorder to avoid water evaporation, pierce a small hole on handi-wrap by needle for air thermal expansion.

   c) **Convert glycogen hydrolysate to glycogen determination solution:**

   For Liver glycogen determination solution, dilute the liver sample 20 times with distilled water

   Volume of distilled water = Sample weight × 20- Sample weight × 4*

   = Sample weight × 16

   *Note: Sampleis 4 times diluted during hydrolysis.

   d) **Determination solution defatting:**

   Add chlorofom to the determination solution in the ratio;

   Determinationsolution volume(mL) :Chloroform volume(mL)=4:1

   Mix thoroughly by vortex,centrifugate at 4000rpm for10minutes, take supernatant for assay (if glycogen content is too high, dilute before assay.)

2. **Operation table**

	Blank (B)	Standard (S)	Sample (T)
Distilled water(mL)	1.0		0.9
0.01mg/mL standard(mL)		1.0	
Determination solution(mL)			0.1
Chromogenic agent(mL)	2	2	2

Mix thoroughly,placeinboiling water bath for 5 minutes,cool to room temperature, transfer in cuvettes of 1cm light path,measure OD values of all tubes at 620nm(adjust zero with the blank tube).

OD Blank = A_B

OD Standard = A_S $\Delta A_S = A_S - A_B$

OD Sample = A_T $\Delta A_T = A_T - A_B$

3. Calculation:

Glycogen content (mg/tissue) = $\Delta A_T \div \Delta A_S \times Sc \times D \times 10 \div 1.11$

Sc	:	Standard concentration (0.01mg/mL)
D	:	Sample dilution (100 for liver; 20 for muscle)
10	:	Dilution during assay
1.11	:	Coefficient of glucose-glycogen coversion (100µg glycogen reacted with anthrone equals to absorbance of111µg glucose reacted with anthrone)