



ISO 13485:2016 ISO 9001:2015

Ver.251201

Glycogen Assay kit

BC097-01 (50Tests/48Samples)

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

1. Product Principle

Under effect of concentrated sulphuric acid, glycogen is dehydrated to furfural derivant, furfural derivant reacts with anthrone to produce blue chemical, use same method to treat glucose standard solution, do quantification by spectrophotometry. Glycogen is very stable in concentrated alkaline solution, so before chromogenic reaction, please place tissue in hot concentrated alkaline solution to destroy other ingredients and keep glycogen.

2. Reagent Preparation and composition

- **Alkaline solution:** 40mL bottle, it can be stored at room temperature or 2~8°C for 6 months.
- **1mg/mL glucose standard stock solution:** 5mL vial, which can be stored at room temperature or 2~8°C for 6 months.
- **0.01mg/mL glucose standard working solution preparation:** 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.
- **Chromogenic agent:** Powder×6 vials, can be stored at room temperature or 2~8°C for 6 months. When use, add 25mL concentrated sulphuric acid, mix sufficiently, this chromogenic agent should be used soon after preparation.

NOTE:

1. Sulphuric acid's concentration must reach 95%~98% (analytical pure), do not open its container for long time or sulphuric acid will be diluted by absorbing water.
2. Container & volumetric cylinder for chromogenic agent preparation must be absolute dry or Reagent 3 can not be dissolved completely.
3. When you prepare chromogenic agent, please pour powder in a flask, add small amount of sulphuric acid (about 10mL), crush powder by glass rod in order to dissolve completely, then add residuary sulphuric acid, mix sufficiently, can be stored at room temperature away from light.

3. Operation procedure

I. Sample pretreatment:

1. Sampling: Take fresh liver or muscle sample, rinse by physiological saline, dry by filter paper, weigh (it is suggested to control sample weight $\leq 100\text{mg}$, do not $> 100\text{mg}$).

2. Hydrolysis: Sample weight (mg): Alkaline solution(μL) = 1:3, add them in test tube, place in boiling water bath, cool in flowing water. (**For example:** If liver sample weight is 75mg, then alkaline solution volume should be 225 μL . If muscle sample weight is 85mg, then alkaline solution volume is 255 μL .)

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

3. Convert glycogen hydrolysate to glycogen determination solution:

Liver glycogen determination solution's concentration is 1%, so added distilled water volume = Liver sample weight $\times 100$ - Liver sample weight $\times 4^* =$ **Liver sample weight $\times 96$**

Muscle glycogen determination solution's concentration is 5%, so added distilled water volume = Muscle sample weight $\times 20$ - Muscle sample weight $\times 4^* =$ **Muscle sample weight $\times 16$**

Note: Sample is 4 times diluted in hydrolysis.

For example, in 1% liver glycogen determination solution preparation, added distilled water volume = $75 \times 96 = 7200\mu\text{L} = 7.2\text{mL}$; in 5% muscle glycogen determination solution preparation, added distilled water volume = $85 \times 16 = 1360\mu\text{L} = 1.36\text{mL}$.

4. Determination solution defatting: Take prepared determination solution (ivory white lipid always floats on upper layer), add a certain amount of chloroform (Determination solution volume(mL): Chloroform volume(mL)=4:1), mix sufficiently by vortex, centrifugate at 4000rpm for 10 minutes, take supernatant for assay (if glycogen content is too high, then please dilute it before assay.)

II. Operation table:

	Blank tube	Standard tube	Sample tube
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 sufficiently, place in boiling 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 by blank tube).

4. Calculation

1. Formula:

$$\text{Glycogen content (mg/gtissue)} = \frac{\text{OD}_{\text{Blank}}}{\text{OD}_{\text{Standard}}} \times \text{content (0.01mg)} \times \text{times before assay}^* \times 10^{**} + 1.11^{***}$$

Note:

*Sample dilution times before assay refer to dilution times in sample pretreatment step 3, it is 100 for liver sample, 20 for muscle sample.

**10 is dilution times during assay.

**1.11 is coefficient of glucose-glycogen conversion, in other words, absorbance of 100µg glycogen reacted with anthrone equals to absorbance of 111µg glucose reacted with anthrone.