May 17, 2019

Yale - Tissue Glycogen

DOI
dx.doi.org/10.17504/protocols.io.y39fyr6

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DOI: https://dx.doi.org/10.17504/protocols.io.y39fyr6

External link: https://mmpc.org/shared/document.aspx?id=201&docType=Protocol

Protocol Citation: John Stack, Gary Cline 2019. Yale - Tissue Glycogen. **protocols.io https://dx.doi.org/10.17504/protocols.io.y39fyr6**

Protocol status: Working
We use this protocol and it's working

Created: March 13, 2019

Last Modified: May 17, 2019

Protocol Integer ID: 21345

Keywords: Tissue Glycogen, tissue glycogen summary, glycogen content within liver, glycogen content, muscle tissue, liver, tissue

Abstract

Summary:

Procedure for determining the glycogen content within liver and muscle tissue.

Materials

MATERIALS

- Perchloric acid (HClO4) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**244252
- Potassium Bicarbonate (KHCO3) **JT Baker Catalog #**3506-1
- Sodium Acetate **Usb (permanently Closed) Catalog #**75901
- Amyloglucosidase **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7420-100MG

Reagent Preparation:

Reagent 1: Perchloric acid ($HClO_4$) 0.9N

5.9mL concentration $HClO_4$ (62%), make up to 100mL in distilled deionized water.

Reagent 2: Potassium Bicarbonate ($KHCO_3$) 1M

1.0g $KHCO_3$, add 10mL of distilled deionized water.

Reagent 3: Acetate Buffer 0.4M, pH 4.8

32.8g Sodium Acetate (MW = 82g/mol), make up to 1000mL in distilled deionized water. Adjust the pH to 4.8 with acetic acid.

Reagent 4: Amyloglucosidase

2 mg/mL acetate buffer. (eg. 100 mg enzyme/ 50mL acetate buffer would be enough to run 100 samples.)

Note:

Sigma-Aldrich, RRID:SCR_008988

Troubleshooting

1 **Homogenization**

1) Obtain samples of around 25mg. Keep samples in liquid nitrogen until ready to weigh.
2) Record weight.
3) Multiply weight of tissue by 5, and add that amount of 0.6N perchloric acid (PCA) to labeled 12×75mm plastic shaker tubes. Keep these tubes on ice.
4) Once done with above, add tissue to plastic homogenizing tubes and homogenize in shaker at 20Hz for 30 seconds. Do not throw out the PCA homogenized mixture until results are calculated.

2 **Glycogen hydrolysis**

5) Add 50µL of PCA homogenate to labeled microfuge tubes.
6) Add 25µL $KHCO_3$ (1M)
7) Prepare fresh Amyloglucosidase solution (Amylogucosidase and Acetate Buffer in a 2mg enzyme /1mL buffer ratio. Pipette 125 µL of fresh amyloglucosidase into each microcentrifuge tube.
8) Incubate samples for 2 hours at 37ºC
9) Centrifuge for 1 minute at 10,000 rpm.

3 **Background (free glucose in tissue homogenate)**

10) While glycogen samples are incubating, centrifuge tubes containing PCA homogenate for 1 minute at 10,000 rpm.
11) Measure the glucose concentration of the PCA homogenate solution (Cobas Mira Glucose method) to obtain the amount of free glucose in each sample.

4 **Glycogen content analysis**

12) Measure the amount of glucose (Cobas Mira Glucose method) in the 2hr-amyloglucosidase digested samples.

5 **Glycogen Concentration Calculation**

13) Glycogen expressed as the gm % glycogen in tissue (i.e.,weight percentage of liver) is calculated as :

$$gram\,\%\,G = \frac{24(glucose\ from\ glycogen) - 6(free\ glucose)}{1000}$$