Tissue Glycogen

Version: 1
Edited by: John Stack, Gary Cline: Yale MMPC Analytical Core

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

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Perchloric acid ($HClO4$)	Sigma-Aldrich	244252
Potassium Bicarbonate ($KHCO_3$)	JT Baker	3506-1
Sodium Acetate	Usb	75901
Amyloglucosidase	Sigma	A7420-100MG

Protocol:

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 12x75mm 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.

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 37˚C
9) Centrifuge for 1 minute at 10,000 rpm.

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.

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

Glycogen Concentration Calculation

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

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

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 (KHCO3) 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.)