

Glycogen Assay

Reference: Passonneau, JV and Lauderdale VR, Analytical Biochemistry 60, 405-412 (1974)



Tissue Preparation:

- 1) Quickly freeze clamp tissue during harvest and store at -80°C
- 2) Powder tissue under liquid nitrogen with tissue pulverizer
- 3) Weigh out ~30 mg (heart or muscle) <15 mg (liver) of powdered tissue on dry ice (record weight)
- 4) Add 1 ml of ice cold 0.3 M PCA and transfer to dounce on ice
 - a) First, cut off tip of 1 ml tip to facilitate transfer
 - b) When adding liquid continuously tap tube to keep powder from clumping
- 5) In 1 ml glass dounce, homogenize tissue ~5 passes for liver 10-20 passes for muscle and heart
- 6) Homogenate may either be assayed immediately or frozen in liquid nitrogen and stored at -80°C

Spectrophotometer Assay: In Eppendorf tubes, add the following (**volume in ml**):

USE NO GLUCOSE SAMPLE TO BLANK SPECTROPHOTOMETER

	Blank	Standard	Sample		
	2X	2X	Blk	1:1	1:2
50 mM Na Ac, 0.02%BSA, pH 5.5	--	--	0.50	--	--
AG Reagent (50 µg/ml)	0.50	0.50	--	0.50	0.50
H ₂ O	0.05	--	--	--	--
Glucose Std (50 µM to 1.6 mM)	--	0.05	--	--	--
PCA:Tissue homogenates	--	--	0.05	0.05	0.025+0.025 PCA

- 1) You will need two tubes for each of the standard curve samples. You will also need three tubes per experimental sample. One for free glucose which will have no AG. Then two for AG digest to free glucose stored as glycogen. Have one sample 1:1 using 50 µl of PCA:Tissue homogenate and 1 with a 1:2 dilution (25µl PCA:Tissue + 25µl 0.3M PCA). This dilution is critical for samples such as liver that may go off the standard curve; otherwise do a duplicate of your sample.
- 2) When adding samples vortex PCA:tissue mixture before each transfer to suspend particulate matter
 - a) Cut off tip of 200λ tip use for all three transfers
- 3) Vortex all tubes, incubate at room tempt. for 2 hours with shaking
- 4) Centrifuge all tubes for 10' at RT after incubation
- 5) Transfer 0.4 ml of the supernatant into centrifuge tube with 0.5 ml of glucose reagent, mix
 - a) Stagger addition ~30" so that you can start reading samples at 15'
 - b) Incubate 15' room temp
- 6) Read at 340 nm (Visible lamp; program 12); results are Abs to be converted to µmol glucose released from glycogen per gram tissue; see spreadsheet. Fill in curve; sample, sample blank abs; sample dilution factor (2 for 1:2, etc.); dilute sample abs; tissue used in 1ml homogenate in mg.

NOTE: If samples are not freeze clamped immediately, if too little tissue, or tissue from depletion experiments are used negative values may occur. Either exclude sample, rerun sample, or in depletion experiments set at 0. Alternatively, for tissue with high levels of glycogen (i.e. liver) or a model with increased storage your value may be above the curve. In this case exclude value and only use the dilution value.

umol/rxn:	0	0.002	0.005	0.01	0.02	0.04	0.08
Std (mM):	BLANK	0.05	0.1	0.2	0.4	0.8	1.6
H ₂ O:	50.0λ	49.6λ	49.1λ	48.2λ	46.4λ	42.8λ	35.6λ
Gluc (5.56 mM):	0.0λ	0.4λ	0.9λ	1.8λ	3.6λ	7.2λ	14.4λ

Solutions:

1. 0.3 M Perchloric Acid (PCA) (STOCK):
Stock is 60% = 9.2 M; 1.96% = 0.3 M (3.27 ml / 100 ml total)

2. 100 mM Na Acetate Buffer, pH 5.5 (STOCK):
90 mM NaAcetate (m.w. = 136.08 g/mol) = 6.12 g/ 500 ml
10 mM Acetic Acid = 0.294 ml of 17 N Glacial Acetic Acid / 500 ml

3. 50 mM Na Acetate, 0.02% BSA, pH 5.5 (MAKE FRESH):

100 mM Na Ac, pH 5.5	25 ml
10% BSA	0.1 ml
H ₂ O	24.9 ml

(For AG and glucose reagents make fresh and only volume needed as to not waste enzyme)

4. Amyloglucosidase Reagent (AG) (MAKE FRESH):
In 50 mM Na Ac, 0.02% BSA, AG, pH 5.5
(Sigma-Aldrich A-7420, 25 mg; bring up at 10 mg/ml; store @ -20°C)
Need final AG concentration of 50 µg/ml
75 µl in 15 ml; 100 µl in 20 ml; 175 µl in 35 ml

5. Glucose Reagent (MAKE FRESH):

<u>Final Conc.</u>	<u>Stock</u>	<u>Vol/10 ml</u>	<u>Vol/25 ml</u>
100 mM Tris/HCl, pH 8.0	1.0 M	1 ml	2.5 ml
0.04% BSA	10%	40 µl	100 µl
0.4 mM NADP	0.1 M	40 µl	100 µl
0.6 mM DTT	1.0 M	6 µl	15 µl
1.0 mM ATP	0.5 M	20 µl	50 µl
2.0 mM MgCl ₂	1.0 M	20 µl	50 µl
G6PDH (1 µg/ml) *	5 mg/ml	2 µl	5 µl
HK (5 µg/ml) **	10 mg/ml	5 µl	12.5 µl

NADP m.w. = 787.4 g/mol

*G6PDH = Boehringer Mannheim #127 671

** HK = Sigma H-5625

5. Stock Standard = 100 mg/dL (5.56 mmol/L) Glucose Standard Solution (Sigma)