Phenol-Sulfuric Acid Method

Steps listed according to Schaubroeck et al. 2022

Helpful paper for figuring out the 'why':

Ingredients list:

- ☐ Tissue samples
- ☐ *Liquid nitrogen*
- ☐ *Mortar and pestle*
- ☐ *1.5mL tubes (prechilled)*
- ☐ 0.5M NaOH (Sodium Hydroxide crystals; Sigma 655104)) = FW: 40g/mol then 5g NaOH in 250mL DI IN THE 4C FRIDGE
- ☐ 9.5% (w/v) Na_2SO_4 (Sodium Sulfate crystals; Sigma S9627)) = 26.24g in 250mL DI SITTING ON SHELF WITH POWDERS HARDY BENCH
- ☐ 200 proof (100%) Ethanol (Fisher A40920) FLAMMABLE DRAWER HARDY LEMA SIDE
- ☐ Parafilm
- ☐ DI water
- ☐ 1M HCl (Hydrochloric Acid; Sigma 9892) 41.67mL HCl in 250mL DI 4C FRIDGE
- ☐ 95-98% sulfuric acid (Sigma 258105) CORROSIVES DRAWER HARDY LEMA SIDE
- ☐ 5% (w/v) phenol (Sigma 242322) = 13.16g phenol in 250mL DI, 5.26g in 100mL -20C Fridge in aliquots
- ☐ Powdered glycogen (bovine liver - for standard) (Sigma G0885) 4C FRIDGE

Tissue Homogenization:

1. Grind 20-30mg of tissue samples to a fine powder using liquid nitrogen, and a mortar and pestle. Eppendorf tubes (1.5mL) were pre-chilled for this
2. Add 200uL of 0.5M NaOH to each sample, before heating samples for 30 minutes at 100C
    a. Hydrolysis of other proteins // digestion of protein material
    b. But it won't destroy glycogen if using the correct concentration
3. Let cool for 10 minutes before adding 50uL of 9.5% sodium sulfate (Na_2SO_4), and 600uL of ethanol. Then centrifuge at 2000g for 10 minutes.
    a. Ethanol causes the precipitation of glycogen
    b. Na_2SO_4 carries down the precipitated glycogen
4. Discard the supernatant and dissolve the pellet in 100uL DI water, and vortex after sealing lid with parafilm
    a. They seal lids with parafilm in case the tubes got warped during the heating process
5. After vortexing, acidify the sample with 5uL of 1M HCl before adding 200uL of absolute ethanol.
    a. Hydrolyzes the isolated glycogen (glycogen to glucose)

6. Vortex this and centrifuge at 2000g for 10 min.
7. Repeat steps 4-6 2 more times (3 total precipitations from this series)
8. Wash pellets with 50uL of absolute ethanol, then let samples dry for 5-10 minutes after this
9. Dissolve pellet in DI water (try 300, **500,** 700 for test run) and vortex after parafilming the lids again

Assay:

1. There may need to be another dilution here, but we should end up with 50uL of sample, regardless of dilution (add DI water to whatever sample volume we choose to get to 50uL)
2. Then add 300uL sulfuric acid followed by 45uL of 5% phenol. USE AUTOPIPETTE
    a. Acid caused heating and dehydrates glycogen down into 5-hydroxymethylfufural
    b. 5-hydroxymethylfurfural reacts with phenol, producing an orange colored solution that can be analyzed with a spectrophotometer
    c. Doing it in this order reduces background noise (less byproducts because of temperature)
    d. Also the heat of dilution method as opposed to heating with a water bath provided more consistent results.
3. Wait 30 minutes and read 280uL of samples at 488nm

Standard Curve:

1. Make a stock solution of bovine liver glycogen: Put 25mg liver in 50mL DI water
2. Label tubes A-F, label 2 per letter
3. Make the following dilutions:
    a. 50uL DI, 0uL glycogen stock
    b. 40uL DI, 10uL glycogen stock
    c. 30uL DI, 20uL glycogen stock
    d. 20uL DI, 30uL glycogen stock
    e. 10uL DI, 40uL glycogen stock
    f. 0uL DI, 50uL glycogen stock

Notes from runthrough #1:

- Make sure to take the phenol out early so it can thaw (while you do dilutions?)
- Do everything under the hood with sulfuric acid
- Could potentially reduce repetitions of 4-6 from homogenization

Runthrough 2: 6/27/25

- Standard looked great (R^2 = .99)! Next week: Try it out with test samples + SC and test out final dilutions: 300, 500, 700uL

Runthrough 3:

- Stick with 500uL final dilution