Glycogen concentration protocol

from Ifremer

*The standard curve of the concentration is linear up to 1 mg/ml*

Glycogen protocol

Prepare KI2CaCl2 solution

* Prepare a saturated solution of CaCl2: about 500g/500 mL water (you can heat it at 50-60°C to dissolve if needed)
* Add 0.26 g iodine to 2.6 g KI in 10 mL distilled water
* To make the KI2CaCl2 dilute 1.92 mL of the iodine solution in 500 mL of the CaCl2 solution

Add 100 mg (or less) lyophilized tissue to 3 mL 15% trichloroacetic acid (TCA)

Sonicate briefly with hand-held sonicator (or can use the tissue homogenizers)

Store 1 hour at 4°C

Centrifuge 10 minutes at 3,000xg

Add 4 mL ethanol to 500 µl of the supernatant

Incubate overnight at 4°C

Centrifuge 30 minutes at 4,000xg

Dissolve the pellet in 200 µl water

test 3 dilutions of 2x, 5x, 10x, and undiluted

Aliquot 20 µl diluted glycogen + 130 µl KI2CaCl2 solution (see above) to a welled plate to put on a plate reader

Read absorbance at 450 nm after 20 minutes at ambient temperature

Use oyster glycogen for standard: Sigma, Glycogen from oyster Type II G8751-5G

Total carbohydrate protocol

Add 100 mg (or less) of lyophilized tissue to 1 mL H2O

Sonicate (as above)

test 3 dilutions 10x, 20x, 30x (in water)

Add 300 µl of diluted carbohydrates to: 600 µl 5% phenol, 3 mL 98% H2SO4

Read absorbance at 490 and 600 nm in a spectrophotometer (use 600 nm is sample has a lot of turbidity)

\*use glass cuvettes if possible because of the acid. If only plastic are available, be sure to remove reagents quickly so as not to erode the plastic.

Use glucose for a standard