GLUCOSE ASSAY PROTOCOL

This assay measures hemolymph glucose via NADH using the following reactions according to the kit:

glucose + ATP →glucose-6-phosphate + ADP Enzyme: Hexokinase (part of Sigma Infinity® Reagent, TR15321)

glucose-6-phosphate + NAD^+ → 6-phosphoguconate + NADH Enzyme: G-6-P dehydrogenase

NADH is produced in an equimolar ratio to glucose, and absorbs at 340 nm. Measuring A340 thus represents the concentration of glucose in the sample.

PROCEDURE

- The day before the assay:
    - Aliquot into a 96-well plate 99 µL/well of Thermo Infinity® Reagent TR15421 into as many wells as needed for the samples and standard. Seal plate with a plate sealer and freeze at -80° C overnight.
        - The reagent is frozen in order to avoid spuriously high sample absorbances resulting from the rapid breakdown of hemolymph trehalose when hemolymph is added to the reagent. Freezing prevents this process, and the readings are thus more robust and accurate.
    - Also place a thermal block at -80° C overnight. This will be used to keep the 96-well plate as cold as possible when the assay is performed.
    - Prepare sufficient quantity of 0.5 mL and 1.5 mL tubes for hemolymph extraction (make a small, very thin slit at the bottom of the smaller tube, place within larger tube, remove caps from both tubes using pliers).
- The day of the assay:
    - Isolate wandering 3rd instar larvae from vials; separate by sex if required. Keep larvae moist in PBS until assay is ready to be performed.
    - Remove the 96-well plate and the block from -80° C. Place the block on a Styrofoam pad, place the plate on the block such that all wells containing the reagent are directly in contact with the block below.

- For each sample, take sufficient larvae for extracting 1 µL hemolymph (~5 larvae fed 0.15M sucrose, 6-8 larvae fed 1M sucrose). Extract hemolymph as follows (these steps must be done quickly; hemolymph is very volatile after extraction):
    - Blot larvae to remove liquid and place each larva in the inner 0.5 mL tube .
    - Bleed the larvae at their anterior end near mouthhooks, pressing with dissecting tweezers.
    - Spin the tube-within-tube on a table-top centrifuge for 2-3 seconds. Hemolymph will collect in the outer tube.
    - Using a 2.5 uL pipettor, pull out 1 uL of hemolymph and carefully add it to the corresponding well in the 96-well plate.
        - Be careful not to scrape the pipet tip onto the frozen reagent.
        - The hemolymph will freeze quickly upon contact with the reagent. Avoid touching the reagent with the tip – take the tip as close to the reagent as possible without touching the reagent, and quickly pipet out the hemolymph.
- After all hemolymph samples are added to the plate, add 1 uL of glucose standards to corresponding wells.
    - 0, 10, 25, 50, 75, 100, 250, 500 mg/dL of glucose
- Incubate the plate at 37 C for 15 minutes.
- Read the plate for absorbance at 340 nm.

NOTES

I can do up to a maximum of 25 samples (i.e. 25 hemolymph extractions) before the reagent begins to melt. Perform the assay only as long as the reagent remains frozen.