# TREHALOSE ASSAY PROTOCOL

This assay measures hemolymph trehalose indirectly via the following reactions:

trehalose + $H_20$ → 2 glucose  Enzyme: Trehalase (Sigma T8778)

glucose + ATP →glucose-6-phosphate + ADP  Enzyme: Hexokinase (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 gives the amount of glucose in the sample.

**PROCEDURE**

- Take sufficient larvae for extracting 2 uL hemolymph (~8 larvae fed 0.15M sucrose, ~11 larvae fed 1M sucrose). Bleed the larvae and extract hemolymph.
- For hemolymph glucose –
    - Take 1 uL hemolymph, add to 99 uL Infinity Reagent in a 96-well plate (pre-plated with reagent and frozen overnight at -80 C.) Incubate ~15 mins at 37 C. Read plate @ 340nm.
    - STANDARDS: 0,10,25,50,100,250,500,1000 mg/dL – add 1uL standard to 99 uL Infinity Reagent.
- For hemolymph trehalose --
    - Take **1 uL** hemolymph, add to 25 uL **0.25M $Na_2CO_3$** in a PCR tube. Incubate at 95 C for 2 hrs (use a thermal cycler with heated lid to avoid evaporation/precipitation in tubes).
    - Bring to room temperature. Add 8 uL **1M acetic acid**. (stock – J.T Baker 9522-03; dilute 1:10). This brings the mixture to pH 5.2.
    - Add **66 uL 0.25M sodium acetate** pH 5.2. This brings the total volume to 100 uL.
    - Aliquot 40 uL of mixture to a new PCR tube. To this tube add 1 uL **trehalase** enzyme.
    - Incubate 12hrs/overnight at 37 C (use thermal cycler with heated lid).
    - Take 10 uL of reaction mixture, add to 90 uL Infinity Reagent. Incubate ~15 mins at 37 C. Read plate @ 340 nm.
    - FOR STANDARDS: 0,25,50,100,200,400,600,800,1000 mg/dL (Trehalose: Sigma T0167)
        - Add **10 uL** standard to 25 uL **0.25M $Na_2CO_3$** in a PCR tube. Incubate at 95 C for 2 hrs (use a thermal cycler with heated lid to avoid evaporation/precipitation in tubes).
        - Bring to room temperature. Add 8 uL **1M acetic acid**. (stock – J.T Baker 9522-03; dilute 1:10). This brings the mixture to pH 5.2.
        - Add **57 uL 0.25M sodium acetate** pH 5.2. This brings the total volume to 100 uL.
        - Aliquot 40 uL of mixture to a new PCR tube. To this tube add 1 uL **trehalase** enzyme.
        - Incubate 12hrs/overnight at 37 C (use thermal cycler with heated lid).
        - Take 10 uL of reaction mixture, add to 90 uL Infinity Reagent. Incubate ~15 mins at 37 C. Read plate @ 340 nm.

Since the hemolymph was diluted 10-fold more than the standard, the final concentration of hemolymph trehalose is 10 * concentration obtained by reading the plate.

**REFERENCE**

Parrou J.L and Francois J. *A simplified Procedure for a Rapid and Reliable Assay of both Glycogen and Trehalose in Whole Yeast Cells*. Analytical Biochemistry 248, 186-188 (1997)