LSOP Title	Enzymatic Sugar Measurements
LSOP No.	LSOP09
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste*
Risk Assessments	
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1.0 Scope

This procedure covers the protocol for measuring enzymatic sugar concentrations

This CSOP does not cover the extraction process for samples or the use of the microplate reader.

2.0 Definitions

G6PDH – Glucose-6-Phosphate Dehydrogenase

HEPES - Sulfonic acid buffering agent

ATP – Adenosine Tri-Phosphate

KOH – Potassium Hydroxide

$MgCl_2$ – Magnesium Chloride

NADP+ - Nicotinamide Adenine Dinucleotide Phosphate

NADPH – Reduced form of Nicotinamide Adenine Dinucleotide Phosphate

HXK – Hexokinase

PGI – Phosphoglucose Isomerase

INV – Invertase

3.0 Materials and Equipment

1. Microplate
2. Pipette & Pipette Tips

3. G6PDH in ammonium sulphate
4. Mix (see appendix for stocks)
5. Sugars (see appendix)
6. Microplate Reader
7. Centrifuge

4.0 Prescribed Actions

1. Prepare fresh: G6PDH grade II *from Roche in ammonium sulphate* [4°C]
    a. To prepare the solution: centrifuge 2 min at 13,400 rpm, discard the supernatant and dissolve the pellet.

2. **Mix for 1 microplate*:*** 15.5 ml HEPES buffer, 480 µl ATP, 480 µl NADP, 80 µl G6PDH grade II (see appendix for stock solutions)

3. Pipette 150 µL of Mix (equivalent to 100 mM HEPES-KOH, pH 6.9, 3 mM $MgCl_2$, 1 mM NADP+, 2.5 mM ATP, and 0.5 U glucose 6-phosphate dehydrogenase) into microtiter plate
4. Glucose, fructose and sucrose are determined by the sequential addition of
    a. 0.9 U hexokinase (yeast)
    b. 0.05 U phosphoglucose isomerase (yeast)
    c. and 10 U invertase (yeast) to the amount of sugars.
5. Pipette 50 µL aliquots of sample extract into each well

    NB: Generally dispensed as duplicates into a microtiter plate
6. Mixed with the reaction mixture, the reduction of $NADP_+$ to NADPH is monitored at 340 nm using a microplate reader.
7. The increase in A_{340} is used to calculate sugar content using a molar extinction coefficient (e) of 6,220 M_{-1} cm_{-1}.

NB: To correct for the technical variation between microplate readers each value was corrected using a glucose standard curve measured on each individual plate.

5.0 Appendix

Stocks:

1. HEPES/KOH 100 mM, $MgCl_2$ 3 mM, pH 7.0 buffer [-20°C]
2. ATP 60mg/ml [-80°C]
3. NADP 36mg/ml [-80°C]
4. HXK: Hexokinase 900 U•mL-1 (*from Roche:* 1500•U mL-1, 120 µl suspension centrifuged, and pellet dissolved in 200 µl 0.1 M buffer) [-80°C]
5. PGI: Phosphoglucose isomerase 52 U•mL-1 (*from Roche:* 175 U•mL-1, 60 µl suspension centrifuged, and pellet dissolved in 200 µl 0.1 M buffer) [-80°C]
6. INV: Invertase (*from Sigma*– lyophilized powder – dissolve as much as possible of the powder in 200 µl 0.1 M buffer)