| LSOP Title | Enzymatic Sugar Measurements |
|---|---|
| **LSOP No.** | LSOP09 |
| **Version** | 1.1 |
| **Location** | UQ Node/Centre-wide |
| **Policy/Procedure Link** | *UQ- Equipment*<br>*UQ -waste* |
| **Risk Assessments** | |
| **Approved by** | *Franziska Fichtner* |
| **Date Approved** | 30/08/2021 |
| **Date Effective** | 03/06/2021 |
| **Next Review Date** | 03/06/2026 |
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## 1.0 Scope

*This procedure covers the protocol for measuring enzymatic sugar concentrations*

*This LSOP 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)