**Harvesting samples**

Grow experimental cultures to saturation. Set aside an aliquot to quantify cell count on a hemocytometer.

For each sample, harvest 2 x 1 ml of saturated culture and spin out @ 13,000 rpm for 1 min (*Saccharomyces*) or 3 min (*Hanseniaspora*). (1 tube will be no enzyme control).

*Note – Perform no enzyme controls on only one sample per species.*

Wash 2x in 1ml ice-cold H2O.

Resuspend pellet in 250 ul of 250mM Na2CO3.

Boil suspension for 3h @ 95°C. Vortex once per half hour.

Vortex vigorously to resuspend debris.

Add 10ul of amyloglucosidase-z to 1 tube per sample.

Incubate O/N at 54°C.

Add 750 ul H2O to each sample.

Freeze or process immediately with Glucose GO Assay Kit (Sigma)

***Media & Reagents***

*Amyloglucosidase-z (store at -20°C) 200 mMNaAcetate*

100 mg α-amyloglucosidase 820 mg NaAcetate

5 ml 200mM NaAcetate 50 ml H2O

HCl to 5.2

**Glucose (GO) Assay (Sigma)**

Prepare standards in test tubes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube** | **ul H2O** | **ml Glucose Standard** | **ug/ml Glucose** |
| BL | 1000 | 0 | 0 |
| St. 1 | 980 | 20 | 20 |
| St. 3 | 960 | 40 | 40 |
| St. 3 | 940 | 60 | 60 |
| St. 4 | 920 | 80 | 80 |

Prepare a test tube for each sample by adding 1ml sample.

Add 2 Assay Reagent to each to all tubes.

Incubate tubes at 37°C for exactly 30 minutes.

Stop reactions by adding 2 ml 6M H2SO4 and gently mixing.

Spec all samples at 540nm.

***Media & Reagents***

*If first run with fresh kit, prepare kit reagents:*

*Glucose Oxidase/Peroxidase (store at -20°C, discard if turbid)*

Break ampule and dissolve in 39.2ml H2O in a foiled 15ml falcon tube.

*o-Dianisidine (store at 4°C)*

Resuspend 5mg content of vial in 1 ml H2O.

Disolve by inverting vial repeatedly. Foil vial.

*To prep Assay Reagent (Can be sotred up to 1 month at 4°C)*

Add 800ul *o*-Dianisidine to the 39.2ml Glucose Oxidase/Peroxidase foiled falcon tube.

Invert several times to mix.

*6M H2SO4*

33.3 ml stock (18M) H2SO4

66.7 ml H2O