**SUGAR AND STARCH PROTOCOL – by Alex Thompson (Adapted from Jessie Godfrey and Pak Chow)**

**88 samples + 1 standard + 1 clear + 5 curve standards = full(ish) 96-well plate**

**PREP BEFORE DAY 1**

1. Weigh ~25 mg/ sample into tapered 1.50 mL ependorfs

**DIGESTING SUGARS🡪DILUTING SUGARS** (Day 1)

1. Set thermomixer to 90˚C
2. Add 1 mL 80% ethanol
3. Vortex gently
4. Into 90˚C thermomixer for 10 minutes**\***
5. Centrifuge @ 2500 RPM for 4 minutes**\***
6. **Pipette 200 uL of supernatant into a new 1.5mL Eppendorf**
7. Dispose of remaining supernatant
8. **Repeat steps 4-6, 2 more times**
9. Set remaining pellet aside to dry overnight on bench. This will be used for starch analysis later.
10. **Pipette 100** μL sugar extract into 900 uL dH2O in new 1.5 mL eppendorf

**RUNNING SUGARS** (will these dilution amounts work?)

1. Make curve standards in glass tubes(?):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| GFG | stock | 80% etoh | dH2O | total (mL) |
| **0** | **0** | **0.15** | **1.35** | **1.5** |
| 50 | 0.075 | 0.15 | 1.275 | 1.5 |
| **100** | **0.15** | **0.15** | **1.2** | **1.5** |
| 150 | 0.225 | 0.15 | 1.125 | 1.5 |
| 200 | 0.3 | 0.15 | 1.05 | 1.5 |
| 250 | 0.375 | 0.15 | 0.975 | 1.5 |

1. Pipette 30 uL of diluted sugar extract & curve into 96-well plate
2. Robot 60 uL of 2% phenol into 96-well plate
3. Robot 150 mL of conc. H2SO4 into 96 well plate (test with 10x robot repeat pipettes)
4. Let cool for 25 minutes – read absorbance at 490nm

**Starch**

1. Make solution of **alpha-amylase** (see “Enzyme Calculations” document)
2. Make solution of **amylloglucosidase** (see “Enzyme Calculations” document)
3. Set thermomixer to 37C
4. Add 100 uL of a-amylase to pellet
5. Add 100 uL of amylloglucosidase to pellet
6. Add 500 uL of NaOAc to pellet
7. Vortex
8. Place in 37C thermomixer for 4 hours
9. Cool samples to room temp, vortex
10. Centrifuge at 2500 for 4 min
11. Create a 10x dilution of 200 uL supernatant w/ 1000 uL dH20
12. Pipette 100 uL of sample into new Eppendorf tubes
13. Add 1 mL of PGO reagent to 100 uL of each sample tube, vortex
14. Let cool in dark for 45 min.

|  |  |  |  |
| --- | --- | --- | --- |
| glucose | stock | dH2O | total |
| 0 | 0 | 1.5 | 1.5 |
| 50 | 0.075 | 1.425 | 1.5 |
| 100 | 0.15 | 1.35 | 1.5 |
| 150 | 0.225 | 1.275 | 1.5 |
| 200 | 0.3 | 1.2 | 1.5 |
| 250 | 0.375 | 1.125 | 1.5 |

π

**RUNNING PLATES** (Day 2 or Day 3, 2-3 hours)

1. Pipette 50μL from STARCH/PGO tubes into 96-well plate
2. Robot 100 uL 75% sulfuric acid into 96-well plate
3. Let cool in dark for 20 mins
4. Load into the spectrophotometer at 525nm