Creating the Calibrators

1) Create a solution of 10ml water and 18.016mg of glucose in a conical tube. This will be known as solution A

2) Create a solution of 10ml water and 18.016mg of fructose in a conical tube. This will be known as solution B.

3) Create a solution of 100ul of solution A and 900ul of water in an Eppendorf tube. This will give us a 1mM Glucose solution known as solution A'.

4) Create a solution of 100ul of solution B and 900ul of water in an Eppendorf tube. This will give us a 1mM Fructose solution known as solution B'.

5) Mix 1000ul of A' and 1000ul of B' together. This will give us a 2ml solution of 500uM G/F, known as solution C in an Eppendorf tube.

6) Mix 1000ul of solution C and 1000ul of water, making a 2ml 250uM G/F solution known as solution D in an Eppendorf tube.

7) Mix 1000ul of solution D and 1000ul of water, making a 2ml 125uM G/F solution known as solution E in an Eppendorf tube.

8) Mix 1000ul of solution E and 1000ul of water, making a 2ml 62.5uM G/F solution known as solution F in an Eppendorf tube.

9) Mix 1000ul of solution F and 1000ul of water, making a 2ml 31.25uM G/F solution known as solution G in an Eppendorf tube.

10) Mix 1000ul of solution G and 1000ul of water, making a 2ml 15.625uM G/F solution known as solution H in an Eppendorf tube.

Protocol

1) Gather the Amylosucrase and .1M Sucrose from a freezer of -20C

2) In Separate Eppendorf tubes:

-50ul of enzyme + 50ul of .1M sucrose creating a solution of .25mg/ml

-30ul of enzyme + 90ul of .1M sucrose creating a solution of .125mg/ml

These will be the master solutions.

3) Incubate both solutions in 37C for 20 minutes

4) Incubate both solutions in 100C for 10 minutes

5) Make the following solutions in microfuge tubes:

-25ul .25 master solution + 25ul of water (2x dilution)

-25ul .125 master solution + 25ul of water (2x dilution)

-10ul .25 master solution +40ul of water (5x dilution)

-10ul .125 master solution + 40ul of water (5x dilution)

6) Create Calibrators of 500uM, 250uM, 125uM, 62.5uM, 31.25uM, and 15.6uM glucose/fructose and a blank of deionized water

7) Add the samples, master solutions, calibrators, and .1M sucrose in water into a 96 well plate in the arrangement shown below. Apply 10ul per well

	1	2	3	4	5	6	7	8	9	10	11	12
A	.25 Master Solution	.25 Master Solution	.25 Master Solution	.25 Master Solution	.125 Master Solution	.125 Master Solution	.125 Master Solution	.125 Master Solution	.1M Suc	500uM	500uM	500uM
B	.25 2x	.25 2x	.25 2x	.25 2x	.125 2x	.125 2x	.125 2x	.125 2x	.1M Suc	250uM	250uM	250uM
C	.25 5x	.25 5x	.25 5x	.25 5x	.125 5x	.125 5x	.125 5x	.125 5x	.1M Suc	125uM	125uM	125uM
D									.1M Suc	62.5uM	62.5uM	62.5uM
E										31.25uM	31.25uM	31.25uM
F										15.6uM	15.6uM	15.6uM
G										Blk	Blk	Blk
H												

8) Using the appropriate multi-pipettes, add 210ul of distilled water, 10ul of solution 1 and 10ul of solution 2 using separate 50ml reservoirs

9) Mix with pipettes and incubate at room temp for 3 minutes

10) Read absorbance using a plate reader at 340 nm

11) Using the appropriate multi-pipettes, Add 2ul of suspension 3 from a 50ml reservoir

12) Mix with pipettes and incubate at room temp for 5 minutes.

13) Read absorbance at 340 nm using a plate reader. If significant difference is noted in between the same samples, re-read every 2 minutes until difference becomes insignificant.

14) Using the appropriate multi-pipette, add 2ul of suspension 4 from a 50 ml reservoir

15) Mix using pipettes and incubate at room temp for 10 minutes

16) Read absorbance using a plate reader at 340 nm

17) Dispose of the plate and materials

Analysis

1) Export the plate readings to MS Excel

2) Find the average values of the blank absorbances for each reading

3) Subtract the average blank absorbance for the second reading by the average blank absorbance for the first reading. This will be the noise value for glucose.

4) Subtract the average blank absorbance for the third reading by the average blank absorbance for the second reading. This will be the noise value for fructose.

5) For each well, subtract the second reading by the first reading, then subtract by the noise value for glucose to get the absorbance values for glucose

6) For each well, subtract the third reading by the second reading, then subtract by the noise value for fructose to get the absorbance values for fructose

7) Plot a calibration curve (at least 5 points) with a line of best fit (absorbance in x-axis, concentration in y-axis). R-squared value ideally in between .98 & .99

8) Use the line of best fit equation to convert each absorbance value into a concentration