## Overview and Considerations:

The analysis principle involves thecondensation of **glucose** **with** a primary aromatic amine in glacialacetic acid, forming an equilibrium mixture of a glycosylamineand the corresponding Schiff base.

## Reagents:

1. Make sure that the 96 well plate adapter is installed in the 85oC heating block;
2. 96 well plate, flat bottom;
3. Sealing tape;
4. Quantichrome kit, stored at room temperature in the pink shelves (Solution area #2);
5. Glucose standards, stored at -20oC (Chemical Fridge 1);
6. Samples include DMEM (1g/L), as well as 0mM, 0.5mM, 2.5mM, 5mM, 7.5mM, 10mM, 25mM. To make 25mM glucose, dissolve 0.227g of glucose in 50mls of water. Use the high accuracy balance to measure out the glucose.
7. Repeating pipet.

## Standards

1. Make at 25mM soution of glucose in water (0.227g in 50mls)
	* Use fresh MilliQ water
2. For 10mls of each standards, make the following dilutions:
	* 25mM: 10mls of stock, 0mls of water
	* 10mM: 4mls of stock, 6mls of water
	* 7.5mM: 3mls of stock, 7mls of water
	* 5mM: 2mls of stock, 8mls of water
	* 2.5mM: 1ml of stock, 9mls of water
	* 0.5mM: 0.2mls of stock, 9.8mls of water

## Sample Prep:

1. Save ~250ul-1ml of cell culture supernatant in a clean 1.5ml ependorf tubes;
2. Snap freeze on dry ice and store at -80oC until required;
3. Thaw rapidly in a water bath;
4. Spin briefly, 5min full speed, in an eppendorf tube to pellet debris;
5. There is not need to transfer the supernatant

## Protocol

1. For each sample we are going to take two measurements;
2. Use a repeating pipett to add 100ul of reagent to an appropriate number of wells;
3. Place 5ul of standard or sample in 2 wells of a 96 well plate (Use the p10 and the high accuracy tips);
4. Seal samples, vortex briefly using the 96 well plate attachment;
5. Spin in centrifuge 1min, 1000xg;
6. Heat to 85oC for 8min;
7. Incubate on ice for 2min;
8. Spin in centrifuge 1min, 1000xg;
9. Read absorbance at 630nM on the 96 well plate reader in WBSB 517.

## Calculations

1. Use the blank to determine background, subtract this from all of the samples;
2. Calculate a standard curve;
3. mM = (OD sample-OD blank)/Slope)
4. To make sure that your calculations are correct, check that you mM concentration is not higher that the low glucose DMEM standard.