

SOP: Glucose Determination Assay Using Spectrophotometry

Approvals:

Preparer: Jason McMillan

Date: 26JUN14

Reviewer: Dr. Maggie Bryans

Date: 27JUN14

1. Purpose:

1.1. Use of the Glucose Determination Assay.

2. Scope:

2.1. Applies to the quantitative determination of Glucose in Conditioned Media.

3. Responsibilities:

3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1. <https://medtestdx.com/product/pointe-glucose-oxidase-open-channel-reagent/>

5. Precautions:

5.1. The reagent should not be used if it has developed turbidity or other signs of microbial growth.

5.2. The reagent should not be used if it fails to meet linearity claims or fails to recover control values in the stated range.

6. Materials:

6.1. P-20 and P-1000 micropipette and tips

6.2. Micro centrifuge tubes

6.3. Timer

6.4. Spectrophotometer able to read at 500nm

6.5. Cuvettes

6.6. Water bath (37°)

6.7. Pointe Scientific Glucose(oxidase) Liquid reagent Catalog# G7521-120

6.8. Pointe Scientific General Chemistry Standards Catalog# G7518STD

6.9. Pointe Scientific General Chemistry Control Level II Catalog# C759150

7. Procedure:

7.1. Running Assay

7.1.1. Turn on water bath and set to 37°C.

7.1.2. Label micro centrifuge tubes “Blank,” Control,” Standard,” “Sample Name #'s.”

7.1.3. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C water bath for 5 minutes.

7.1.4. Remove micro centrifuge tubes from the water bath.

7.1.5. Add 10µl of control solution to the “Control” tube, 10µl of Glucose standard to the “Standard” tube, and 10µl of sample to each of their respective “Sample” micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.

7.1.6. Place all of the micro centrifuge tubes except for the “Blank” micro centrifuge tube back into the 37°C water bath for 10 minutes.

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- 7.1.7. Remove the micro centrifuge tubes from the 37°C water bath and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.
- 7.1.8. Read and record the absorbance of the tubes at 500nm using the “Blank” tube to zero the spectrophotometer.
- 7.1.9. Record absorbance values for each of the tubes and calculate the concentration of Glucose.

7.2. Calculate Glucose Concentration.

- 7.2.1. Formula to determine glucose concentration:

$$\text{Glucose (mg/dl)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mg/dl)}$$

8. History:

Name	Revision	Date	Amendment
Jason McMillan	0	26JUN14	Initial release
Hetal Doshi	1	02AUG2021	Updated website link and added catalog number for the reagents