# PHENOL-SULFURIC ACID MANNOSE DETECTION ASSAY

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## BACKGROUND AND NOTES

- This assay is sensitive to mannose from 1 – 100 $\mu$g but is sensitive towards other carbohydrates
- This assay was used for the quantification of complex carbohydrates that constitutes bacterial cell wall
    - Whole Gram-positive bacterial lysates can be detected using this assay providing a fast, colorimetric alternative to bacterial quantification
- Volumes suggested below can be manipulated but the **ratio of reagents must be maintained**

## EQUIPMENT

- Equipment:
    - Spectrophotometer capable of measuring absorbance at 490nm
    - 96-well plate
- Materials:
    - Concentrated $H_2SO_4$
    - 5% (w/v) Phenol

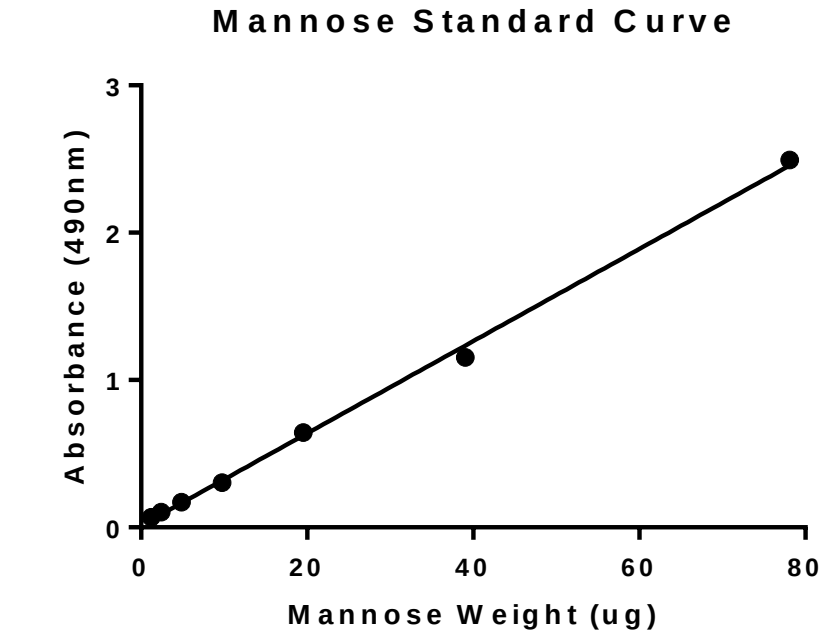
## PROTOCOL

- Preparatory Work:
    - Create a 5% w/v phenol in water solution; small volumes of phenol are required per sample but a stock solution may be made for convenience
        - Phenol is primarily non-polar but at low concentrations, it is soluble in water
    - The following steps can be done right in a 96-well plate or in Eppendorf tubes; absorbance measurements are still to be done in a plate reader

1. Add 50 ul of sample into 96 well plate
    a. It is best to create triplicates per measured sample
    b. Ensure the sample vehicle does not contain sugars
2. **Forcible and quickly** eject 150ul concentrated $H_2SO_4$ into each well
    a. **This reaction is highly exothermic; be careful of fumes and heat**
    b. Quickly ejecting into each well helps with mixing
3. Gently shake the plate until solution looks homogenized
    a. Sugars present in the sample will react with the acid to quickly change into a yellow-brown colour
4. Add 30ul of 5% phenol
5. Let stand for 10 minutes at room temperature

6. Read absorbance at a wavelength of 490 nm

## RESULTS




$$Y = 0.03128*X + 0.01288$$

$$R^2 = 0.9981$$

**Figure #1: Phenol-Sulfuric Acid Mannose Detection Assay Standard Curve.** The standard curve displays weight of mannose detected. All values can be considered in concentrations by accounting the 50 $\mu$l sample volume.

## LINKS AND REFERENCES

1. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S-I, Lee YC. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Analytical Biochemistry. 2005 Apr;339(1):69–72.