



METHYLENE GREEN SDS DETECTION ASSAY

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BACKGROUND

- SDS is a common contaminant in many procedures used in molecular biology, and ensuring that a sample is SDS-free can often be critical to the outcome of an experiment
- The methylene green SDS detection assay utilizes the partitioning of methylene green ionically bound to the dodecyl sulfate chain from aqueous to organic phase to determine SDS concentration.
- This assay was originally adapted to be used with methylene blue¹ however; methylene green is an acceptable replacement compound for the detection and quantification of sodium dodecyl sulfate (SDS) in solutions

EQUIPMENT

- Equipment:
 - o Spectrophotometer that can read cuvettes
 - o Chemical fume hood
 - o Centrifuge capable of spinning 15 mL conical tubes
 - o Vortex
- Materials:
 - o Chloroform
 - o Methylene Green
 - o 0.7 mM Tris-HCl (pH 7.0)

PROTOCOL

- Preparatory Work:
 - o Create a 0.5% Methylene Green solution in dH₂O (5 mL)
 - Dilute 0.5% methylene green solution 100x with Tris-HCl (pH 7.0)
- 1. Collect 1 mL of sample and place into 15 mL conical tube
- 2. Add 0.5 mL of 100x diluted Methylene Green solution to each sample
- 3. Add 3 mL of chloroform in fume hood to each sample
- 4. Vortex for 7 seconds
- 5. Centrifuge at room temperature at 2,000 rpm for 3 minutes
 - a. Be gentle after centrifugation to maintain the two distinct phases
- 6. Let stand for 10 minutes at room temperature
- 7. Aliquot 1 mL of the sample chloroform phase to a cuvette
- 8. Read cuvette in spectrophotometer at a wavelength of 659 nm
 - a. Use a methylene green + water as a blank
- 9. Determine concentration of SDS using the standard curve in **Figure 1**

RESULTS

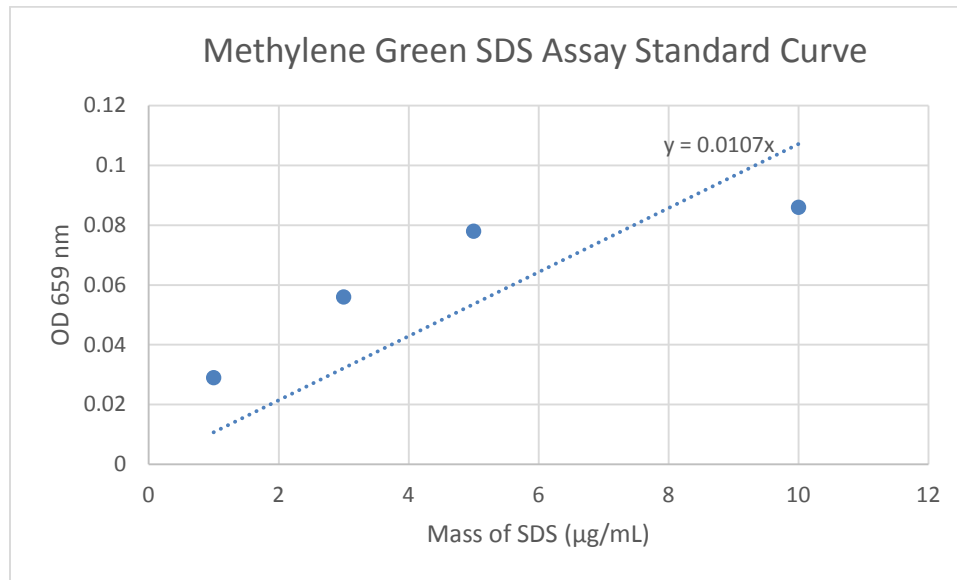


Figure #1: Methylene green SDS detection assay standard curve. Standard curve to be used with the methylene green assay method of quantifying contaminating SDS in solution. Created using a methylene green SDS assay of known concentrations of SDS solution.

LINKS AND REFERENCES

1. Hayashi K. A rapid determination of sodium dodecyl sulfate with methylene blue. *Anal. Biochem.* 67(2): 503-506, 1975. Pubmed: 1163770