METHYLENE GREEN SDS DETECTION ASSAY

Name: Jason Fan and Charles Yin
Date: August 8, 2012
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

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 blue\(^1\) however; methylene green is an acceptable replacement compound for the detection and quantification of sodium dodecyl sulfate (SDS) in solutions.

EQUIPMENT

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

PROTOCOL

- Preparatory Work:
  - Create a 0.5% Methylene Green solution in dH\(_2\)O (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
    - 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
    - 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