Lead Assay Protocols

Experiment: Lead Assay

Materials:

- 20nm Gold Nanoparticles
- Lead Nitrate
- GSH
- 480 µL of L-Glutathione
- NaCl (1 M)
- Phosphate Buffer (50 mM, pH=7)
- 65.82 g/L of Sodium Monobasic
- 93.1 g/L of sodium dibasic
- D.I Water
- LB
- MRS
- 15 mL conical tubes
- 1.5 centrifuge tube
- Plate reader

Prepare Lead Spiked Water Stock

1.) Measure out 100 mg of Lead nitrate to get 1000 ppb

2.) Dilute as necessary to achieve concentrations of 5 ppb, 10 ppb, 15 ppb, 30 ppb, 50ppb, and 100 ppb.
   5 ppb - 1 µL Pb in 20 mL of D.I
   10 ppb - 1 µL Pb in 10 mL of D.I
   15 ppb - 1 µL Pb in 6.67 mL of D.I
   30 ppb - 1 µLL Pb in 3.3 mL of D.I
   50 ppb - 1 µL Pb in 2 mL of D.I
   100 ppb - 1 µL Pb in 1 mL of D.I

3.) For Media:
   50mL Media
   49mL Distilled H_2O (50mL Distilled H_2O for control)
   + 1mL Pb-Spiked H_2O (None for control)

   100mL total
   ---->50mL x 1g/mL x .001kg/g = 0.05kg

LB:

25g Agar powder/ 1L H_2O = 1.25g Agar/50mL H_2O -> 51.25g or .05125kg
Mass solution= .10125kg = .10125mg Pb for 1ppm or 1000ppb
5000ppb => .10125mg x 5 = 5.065mL
2000ppb => .10125mg x 2 = 2.026mL
1500ppb => .10125mg x 3/2 = 1.5195mL

1000ppb => .10125mg x 100mL/10mg = 1.013mL
750ppb => .10125mg x 3/4 = .0759mg = .759mL = 759µL
500ppb => .10125mg x 1/2 = .050625mg = 506µL
250ppb => .10125mg x 1/4 = .0253125mg = 253µL
100ppb => .10125mg x 1/10 = .010125mg = 101µL
50ppb => .10125mg x 1/20 = .0050625mg = 50.6µL
25ppb => .10125mg x 1/40 = .00253125mg = 25.3µL
15ppb => .10125mg/ 66.67 = .0015187mg = 15. 2µL
10ppb => .10125mg x 1/100 = .0010125mg = 10.13µL
5ppb => .10125mg x 1/200 = .00050625mg = 5.06µL

MRS:

55g MRS/ 1L H_2O = 2.75g MRS/ 50mL H_2O -> 52.75g or .05275kg
Mass solution= .10275kg = .10275mg Pb for 1ppm
5000ppb => 5.14mL
2000ppb => 2.056mL
1500ppb => 1.542mL
1000ppb => 1.028mL
750ppb => 770.6µL
500ppb => 513.8µL
250ppb => 256.7µL
100ppb => 102.8µL
50ppb => 51.4µL
25ppb => 25.7µL
15ppb => 15.4µL
10ppb => 10.28µL
5ppb => 5.14µL
For Volumes of 100mL

Standard Curve: Well Preparation and Plate Reading

4.) In wells A1-A7, place samples 5 ppb-control
5.) In each well add 41.5 µL of the lead spiked water, 10.5 µL of dH_2O, 34.6 µL of AuNP, and add 1.5 µL of GSH. The order and timing is very important. This order produces the greatest difference between the absorbance with and without lead present. If possible, use a multichannel pipette to eliminate timing errors. For other types of media:

LB: In each well add 41.5 µL of the lead spiked LB, 2 µL of LB Control, 34.6 µL of AuNP, and add 10 µL of GSH. The control with GSH is 43.5 µL and 10 µL of GSH. The control without GSH is 53.5 µL.

MRS: In each well add 41.5 µL of the lead spiked MRS, 4 µL of MRS Control, 34.6 µL of AuNP, and add 8 µL of GSH. The control with GSH is 45.5 µL and 10 µL of GSH. The control without GSH is 53.5 µL.

6.) Place replicates in B1-B7, C1-C7, and D1-7
7.) Place in plate reader at Absorbance A_{595} (due to the available wavelengths on plate reader, ideally around A_{610}, but a ratio of two different wavelengths can also be used to limit error). Read at specific time points depending on media:

LB: min, optimum at ~
MRS: min, optimum at ~
Water: Before 14:00 min