Lowry Protein Assay

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METHOD 1 – 96 Well Plate

Materials

- Modified Lowry Protein Assay Kit (contents: Modified Lowry Protein Assay Reagent, Folin & Ciocalteu's Phenol Reagent, Albumin Standard, ampules) (From Kit: Thermo Scientific cat# 23240)
- 2 mL test tubes (10)
- Elution buffer
- Autoclaved water
- COSTAR (#3595)96 well plate and cover
- Multi-channel pipette
- Spectrophotometer

Methods

NOTE: Do not use Sarstedt Plates

1. Use one Albumin Standard (2.0 mg/mL) as stock and use Elution Buffer as Diluent

Prepare the following BSA Standards Accordingly. Store in -20°.

Vial (name)	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	125 µL	375 µL of stock	1,500 µg/mL
B	312.5 µL	312.5 µL of stock	1,000 µg/mL
C	155 µL	155 µL of vial A dilution	750 µg/mL
D	312.5 µL	312.5 µL of vial B dilution	500 µg/mL
E	312.5 µL	312.5 µL of vial D dilution	250 µg/mL
F	312.5 µL	312.5 µL of vial E dilution	125 µg/mL
G	400 µL	100 µL of vial F dilution	25 µg/mL
H	400 µL	100 µL of vial G dilution	5 µg/mL
I	400 µL	100 µL of vial H dilution	1 µg/mL
J	500 µL	0	0 µg/mL = blank

2. Prepare the Folin-Ciocalteu Reagent by diluting the 2X supplied reagent 1:1 with autoclaved water. Each test replicate requires 20 µL of 1X Folin-Ciocalteu Reagent when used with a 96-well plate. The diluted reagent is unstable and must be made on the same day as use
3. Pipette 40 µL of each standard and unknown sample into a separate well
4. Add 200 µL of Modified Lowry reagent to each well at nearly the same moment using a multi-channel pipette
5. Immediately mix microplate with spectrophotometer for 30 seconds
6. Cover the plate with plastic lid and incubate at room temperature for exactly ten minutes
7. Add 20 µL of 1X Folin-Ciocalteu Reagent to each well using a multi-channel pipette. Immediately mix with spectrophotometer for 30 seconds
8. Cover plate with plastic lid and incubate at room temperature for 30 minutes
9. Measure the absorbance at 750 nm on a plate reader. Subtract the average 750 nm absorbance value on the Blank standard replicates from the 750 nm value of the other individual standards and unknown sample replicates

10. Prepare a standard curve by plotting the average Blank-corrected 750 nm values for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration for each unknown sample.

METHOD 2 – Cuvettes

Materials

- Modified Lowry Protein Assay Kit (contents: Modified Lowry Protein Assay Reagent, Folin & Ciocalteu's Phenol Reagent, Albumin Standard, ampules)
- 2 mL test tubes (10)
- Elution buffer
- Autoclaved water
- Polystyrene Cuvettes
- Spectrophotometer

Methods

1. Use two Albumin Standard (2.0 mg/mL) as stock and use Elution Buffer as Diluent

Prepare the following BSA Standards Accordingly. Store in -20°.

Vial (name)	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	250 µL	750 µL of stock	1,500 µg/mL
B	625 µL	625 µL of stock	1,000 µg/mL
C	310 µL	310 µL of vial A dilution	750 µg/mL
D	625 µL	625 µL of vial B dilution	500 µg/mL
E	625 µL	625 µL of vial D dilution	250 µg/mL
F	625 µL	625 µL of vial E dilution	125 µg/mL
G	800 µL	200 µL of vial F dilution	25 µg/mL
H	800 µL	200 µL of vial G dilution	5 µg/mL
I	800 µL	200 µL of vial H dilution	1 µg/mL
J	1000 µL	0	0 µg/mL = blank

2. Prepare the Folin-Ciocalteu Reagent by diluting the 2X supplied reagent 1:1 with autoclaved water. Each test replicate requires 100 µL of 1X Folin-Ciocalteu Reagent when used with cuvettes. The diluted reagent is unstable and must be made on the same day as use
3. Pipette 50 µL of each standard and unknown sample into a separate well
4. Add 1 mL of Modified Lowry reagent to each cuvette at 15-second intervals. Mix well and incubate at room temperature for exactly 10 minutes.
5. Maintain the 15-second interval between cuvettes by adding 100 µL of 1X Folin-Ciocalteu Reagent to each cuvette exactly at the end of each cuvette's 10-minute incubation period. Mix well.
6. Cover and incubate at room temperature for 30 minutes
7. Measure the absorbance at 750 nm with a spectrophotometer. Zero using a cuvette filled with only water. Subtract the average 750 nm absorbance value on the Blank standard replicates from the 750 nm value of the other individual standards and unknown sample replicates
8. Prepare a standard curve by plotting the average Blank-corrected 750 nm values for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration for each unknown sample.

Reagents

Elution Buffer, 250 ml
In 250 ml dH_2O
+ 1.73 g $NaH_2PO_4 \bullet H_2O$, 50 mM (FW 137.99 g/mol)
+ 4.39 g NaCl, 300 mM (FW 58.44 g/mol)
+ 4.25 g Imidazole, 250 mM (FW 68.08 g/mol)
pH to 8.0 using NaOH store at 4°C

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