

PROTEINS (LOWRY) PROTOCOL

1. INTRODUCTION

The "Lowry Assay: Protein by Folin Reaction" (Lowry *et al.*, 1951) has been the most widely used method to estimate the amount of proteins (already in solution or easily-soluble in dilute alkali) in biological samples. First the proteins are pre-treated with copper ion in alkali solution, and then the aromatic amino acids in the treated sample reduce the phosphomolybdatephosphotungstic acid present in the Folin Reagent. The end product of this reaction has a blue color. The amount of proteins in the sample can be estimated via reading the absorbance (at 750 nm) of the end product of the Folin reaction against a standard curve of a selected standard protein solution (in our case; Bovine Serum Albumin-BSA- solution). Please refer to Sections 22.7.5 and 22.7.6 at "*Methods for General and Molecular Bacteriology*" by Gerhardt *et al.* (1994) for further information and useful tips. We have this book at the Raskin Lab (Loc: 4217).

2. MATERIALS and CHEMICALS

- Clean Glassware
- Glass tubes : of any kind, approx. 10 mL
- Semi-micro cuvettes : Standard type plastic disposable cuvettes with 10 mm path length, sample capacity of 1.5-3 mL (FisherBrand; 14-385-942): Different than the total carbohydrates measurements, we have to use these semi-micro cuvettes, since the volume of the end product we need to measure is only 1.3 mL.
- Spectrophotometer : that goes up to 750nm (Loc: 4215).
- Bovine Serum Albumine : Albumin bovine serum, Cohn fraction V (Sigma-Aldrich; A9647) (Loc: 4217, +4oC small fridge)
- Folin Reagent : Folin and Ciocalteu's Phenol Reagent, 2N, Sigma (Loc: 4130)
- Sodium tartrat : Na₂Tartrat.2(H₂O) (Loc: 4130)
- Copper sulfate : CuSO₄.5(H₂O) (Loc: 4130)
- Other chemicals : NaOH, Na₂CO₃

3. PREPARING the SOLUTIONS

- The Lowry solution is a mixture of the above chemicals, except the Folin Reagent. The Lowry solution should be prepared fresh, at the day of measurement. Though the individual solutions for the Lowry solution can be prepared in advance and then mixed at the day of measurement. Personal note: Different than the protocol given by Gerhardt *et al.* (1994), I dissolve CuSO₄.5(H₂O) and Na₂Tartrate.2(H₂O) separately. Please compare this protocol and the one given by Gerhardt *et al.* (1994). You can find my calculations for this comparison at the end of this protocol. Use ddl water.
- Solution A is a dilute alkali solution. 2N Folin and Ciocalteu's Phenol Reagent contains hydrochloric and phosphoric acids. So, wear gloves, goggles, lab coat, and work carefully

SOLUTION A (alkaline solution) (for 500 mL)

2.8598 g NaOH
14.3084 g Na₂CO₃

SOLUTION B (for 100 mL)

1.4232 g CuSO₄.5(H₂O)

SOLUTION C (for 100 mL)

2.85299 g Na₂Tartrate.2(H₂O)

LOWRY SOLUTION (fresh; 0.7 mL/ sample)

Solution A + Solution B + Solution C with a ratio (vol:vol) of 100:1:1.

FOLIN REAGENT (instant fresh; 0.1 mL/ sample)

5 mL of 2N Folin and Ciocalteu's Phenol Reagent + 6 mL of ddl water

- This solution light-sensitive. So it should be prepared at the last 5 min of the first sample incubation (see the procedure) and kept in an amber container.
- At the protocol given by Gerhardt *et al.* (1994), the dilution ratio for the Folin and Ciocalteu's Phenol Reagent is 1:1, resulting in a 1N Folin reagent.

BSA STANDARD PROTEIN SOLUTION (fresh)

Although BSA is a water-soluble protein, it takes time to dissolve it completely. So, prepare this stock solution and keep it mixed i.e., for 1 hour before starting the experiments.

- Weigh 0.05 g of BSA and add to a 500 mL volumetric flask containing ddl water
- Stir well to dissolve and adjust the volume to 500 mL with ddl water: Final concentration of the stock is 100 mg BSA/L.
- Prepare dilutions* in 15 mL Cricing tubes, following the recipe at [Table 3.1](#).

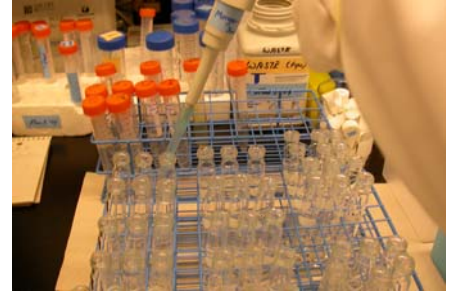
Table 3.1 Dilutions from the BSA stock solution (100 mg/L) for the standard curve

V ddl water	V of stock BSA solution	Final conc.
10 mL	0 mL	0 mg/L
8 mL	2 mL	20 mg/L
6 mL	4 mL	40 mg/L
4 mL	6 mL	60 mg/L
2 mL	8 mL	80 mg/L
0 mL	10 mL	100 mg/L

***POINTS OPEN FOR DISCUSSION:** I've been preparing standard curves at the above range for my measurements. I do not need to dilute the BLANK and SUPERNATANT samples from the EPS extraction experiments, but I usually need to dilute my DOWEX-extracts (1:5 to 1:10 dilutions). Depending on the general character of the sample and the expected protein values for the supernatant and the DOWEX-extract, a higher-range calibration curve can be prepared to avoid sample dilution. Also at the protocol given by Gerhardt *et al.* (1994), the concentration of the stock standard solution is given as 500 mg/L, instead of 100 mg/L. Though since we're preparing the stock solution fresh every time, I think we can continue to prepare a stock solution of 100 mg/L, if we continue to use the above range for the standard curve.

4. PROCEDURE

- **Wear gloves, goggles, lab coat, and work very carefully**
- Take your samples out of -20°C freezer to thaw
- Prepare the **stock BSA solution and dilutions** for the standard curve (Table 3.1, prepare **freshly**)
- Prepare the Lowry solution by mixing the Sol.A, B, and C.
- When totally thawed, vortex well and dilute your samples with ddH₂O if necessary.
- Work in triplicates
- Vortex your **sample/dilution (or standard)** well to mix and transfer **0.5 mL** to a 10 mL glass tube. You can use a 1000 uL pipetman at this step
- **Add 0.7 mL of Lowry Solution** to the tubes. You can use a 1000 uL pipetman.
- **Cap** and **vortex** briefly to mix (decrease the speed of the vortex to approx. 3-4)
- Incubate for **20 min at room temp. in dark**.
- At the last 5 minutes, prepare the diluted Folin Reagent.



- After 20 min of incubation, take the samples out and **add 0.1 mL of diluted Folin Reagent** to each tube.

- **Cap** and **vortex immediately** to mix.



- Incubate once more for **30 min or longer at room temp. in dark**.
- While waiting, turn on the UV-VIS spec to warm up and stabilize (Loc.:4215)
- Before using the UV-VIS spec, please get the general training from the Lab. Manager.
- After 30 min, vortex the samples briefly
- Transfer the samples (1.3 mL) to semi-micro disposable cuvettes



- Working at the **Quantitative** mode and **@750 nm**, auto-zero the spec. with 2 semi-micro cuvettes filled with ddH₂O. Then record the absorbance values for the standards (standards mode) and the samples (unknown mode).
- Prepare the calibration curve from the abs. readings of the standards and calculate the protein content of the samples in mg BSA/L from this curve.

5. CLEAN-UP and WASTE DISPOSAL



- When done with the spec. readings, collect all the samples (in the semi-micro cuvettes), left-over Lowry and Folin solutions in containers; label
- Collect the emptied semi-micro disposable cuvettes in a plastic bag; label
- Collect all the generated waste to a designated area to be picked-up.

- Get a CWM-TRK-01 form (Request for Pickup of Chemical Waste) from the Lab. Manager and fill it out for the liquid and solid wastes to be picked up and disposed. You can find the copies of the forms I filled out either at Shaoying Qi's office or in the folder I left to Eberhart Morgenroth and Julie Zilles.
- Rinse all the glassware and then clean with soapy water, rinse well again
- Clean the work space well

6. NOTES

- Different proteins give different responses to the Lowry method. So pick a particular commercially available protein as the standard and keep using it for all of your experiments.
- It's advised to run the samples in duplicates (Gerhardt *et al.*, 1994), though I run them in triplicates.
- Always run blanks. In our case, the blank is the first sample of the standard curve (0 mg BSA/L).
- Please refer to section 22.7.5.b (2nd paragraph) at Gerhardt *et al.* (1994) for the additional treatment of the samples containing proteins which are not soluble in dilute alkali.
- Commonly used detergents, reducing agents, buffers, ammonium sulfate > 0.15%, 1-5M of many salts, some sugars, glycine, Tris buffer, some chelating reagents, compounds with sulfhydryl groups, amino acids, phenol, usually interfere with the Lowry assay.

7. REFERENCES

Gerhardt, P.; Murray, R.G.E.; Wood, W.A.; Krieg, N.R. (1994) "*Methods for General and Molecular Bacteriology*", ASM, Washington DC, ISBN 1-55581-048-9, p 518.

Frolund, B.; Palmgren, R.; Keiding, K.; Nielsen, P.H. (1996). "Extraction of extracellular polymers from activated sludge using a cation exchange resin", *Water Res* **30**(8), pp. 1749-1758.

Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. (1951) "Protein measurement with the Folin Phenol Reagent", *J Biol Chem* **193**, pp. 265-275.

8. CALCULATIONS

The recipes for the solutions given by Gerhardt *et al.* (1994) and the ones presented in this protocol seem to be different. Though, when the amounts of chemicals in the final reaction volume are calculated, it's clear that the differences are not significant.

Gerhardt <i>et al.</i> (1994)		This protocol	
Reagent A		Solution A	
4 g in 1 L	0.1 N	NaOH	2.8598 g in 500 mL 0.143 N
20 g in 1 L	0.1 N	Na ₂ CO ₃	14.3084 g in 500 mL 0.135 N
Reagent B		Solution B	
0.5 g in 100 mL	0.02 M	CuSO ₄ .5(H ₂ O)	1.4232 g in 100 mL 0.057 M
1% (w:v)	0.052 M	Na ₂ Tartrate	--
		Solution C	
		Na ₂ Tartrate.2(H ₂ O)	2.85299 g in 100 mL 0.124 M
Reagent C		Lowry Solution	
50 mL ReagA + 1 mL ReagB		100+1+1 mL SolA+SolB+SolC	
0.1N		NaOH	0.14N
0.1N		Na ₂ CO ₃	0.132N
0.0004M		CuSO ₄ .5(H ₂ O)	0.00056M
0.001M		Na ₂ Tartrate.2(H ₂ O)	0.00121M
Reagent D		Folin Solution	
1:1 dilution	1 N	5:11 dilution	0.83 N
Reaction recipe:			
0.2 mL sample +1 mL ReagentC + 0.1 mL ReagentD = 1.3 mL		0.5 mL sample + 0.7 mL Lowry Sol. + 0.1 mL Folin Sol. = 1.3 mL	
Amounts in the final reaction volume of 1.3 mL			
0.077 N		NaOH	0.074 N
0.077 N		Na ₂ CO ₃	0.071 N
0.000308 M		CuSO ₄ .5(H ₂ O)	0.000302 M
0.00077 M		Na ₂ Tartrate.2(H ₂ O)	0.00065 M
0.077 N		Folin C.Phenol Reag.	0.064 N