INSTRUCTION MANUAL

Lowry Assay Kit

Kit for Protein Quantification

(Cat. No. 39236)

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1. Lowry Assay Kit

1.1. General Information

Lowry's method for protein estimation is a most widely acceptable method for accurate determination of protein concentration. SERVA Lowry Assay Kit is based on this method. It is a ready to use reagent kit for rapid estimation of protein with ease and consistancy.

The method is based on combination of biuret reaction and Folin-Ciocalteau reaction. In the first step of the reaction protein binds to copper in alkaline medium and produces Cu ions. In the second step Cu ion catalyzes oxidation of aromatic aminoacids by reducing phosphomolybdotungstate to heteropolymolybdenum blue. This reaction produces strong blue colour which predominantly depend upon tyrosin and tryptophan content of protein and to a lesser extent cysteine and other residues in protein.

1.2. Kit Components

Component	250 Assays
Protein Standard, BSA	3 x 5 mg
Solution A	2,5 ml
Solution B	250 ml
Solution C	30 ml

1.3. Additionally required equipment

- Vortex mixer
- Photometer suitable for measurement at 660 nm wavelength and the usage of microcuvettes
- Plastic cuvettes

1.4. Storage conditions

The recommended storage temperature for the Lowry Assay Kit is +2 – 8 °C. Under these storage conditions the unopened reagent is at least useable until: see expiry date on the label.

2. Procedure of the Lowry Assay

2.1. Prepration of Standards and Reagents

2.1.1. Standard Protein

Reconstitute one vial containing 5 mg BSA with 1 ml distilled water to get 5 mg/ml. This is stable at 4 °C for two weeks.

Dilute 0.2 ml of 5 mg/ml BSA solution with 0.8 ml distilled water to get 1.0 mg/ml just before use.

2.1.2. Complex forming reagent

To one volume of Solution A add 100 volumes of Solution B just before use.

2.1.3. Solution C

Solution C is ready to use.

2.2. Preparation of BSA Reference Solutions

BSA [µg/tube]	V(BSA solution 1 mg/ml) [µl]	V(Diluent) [µl]
0	0	1000
10	10	990
50	50	950
100	100	900
250	250	750
500	500	500

2.3. Protein Quantification Procedure

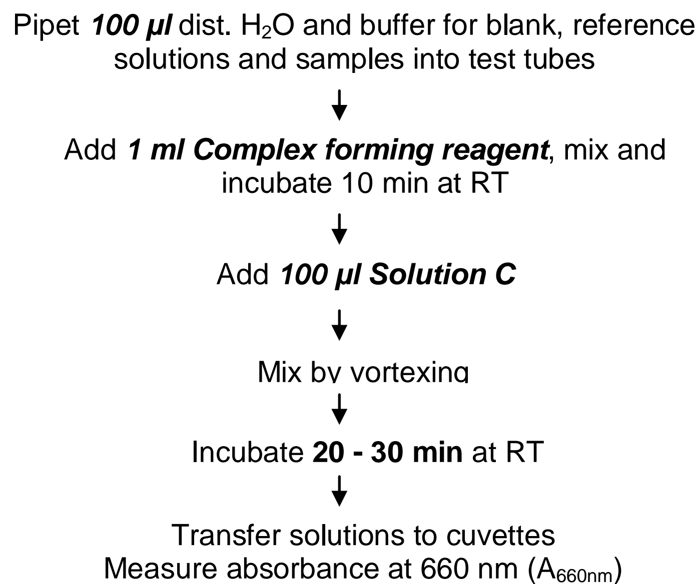
Presence of the following compounds in the sample is known to interfere in the assay and should be avoided.

- Substances containing amine group.
- High molarity buffers of low pH or strong acids. The pH of reaction mixture should be in the range of 10 - 10.5.
- Detergents
- Ammonium sulfate > 3 %
- Sodium phosphate > 0.2 M
- Cesium bicarbonate

Protocol:

- Pipette standard BSA 1 mg/ml, samples and distilled water referring to the protocol given in the following table and adjust the volume to 0.1 ml.
- Add 1 ml complex forming reagent, mix and keep for 10 minutes.
- Add 0.1 ml of Solution III with vortexing and incubate for 20 - 30 minutes.
- Read the optical density on spectro-photometer at 660nm or on a colorimeter using suitable filter.

Flowchart:



2.4. Calculation of Protein Concentrations

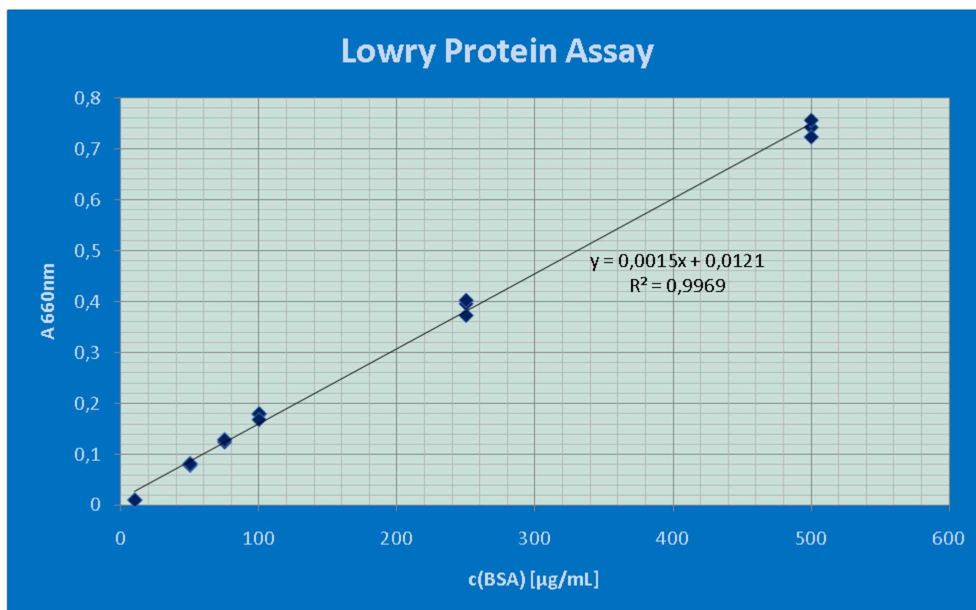
Create a table with the absorbance results obtained from the assay. From the values obtained for the BSA reference solutions create a calibartion curve which is used to determine the protein concentration in the unknown sample.

Table 1 shows exemplary absorbance results for creation of the BSA calibration curve (samples were solved in water) and **Graph 1** the consequentially resulting calibration curve.

c(BSA) [µg/mL]	A_{660nm}
0	0
0	0
0	0
10	0.01
10	0.01
10	0.01
50	0.078
50	0.082
50	0.081
75	0.129
75	0.124
75	0.128
100	0.178
100	0.18
100	0.168
250	0.373
250	0.396
250	0.403
500	0.743
500	0.757
500	0.724

Note:
The data of these BSA references should not be used as a replacement of a calibration curve. The absorbance of the BSA reference solutions in each assay will differ from those presented here.

Table 1: Example for assay data table of BSA reference solutions.



Graph 1: BSA calibration curve produced from the assay data in Table 1.
The data are fit with a linear regression by the line y = 0,0015 x + 0,0121 with an R^2 value of 0.9969.

- Construct a calibartion curve by plotting optical density reading on y axis against standard protein amount (µg/tube) on the x axis
- Record the value x for the protein amount per tube from the calibration curve corresponding to the optical density reading for inividual sample.
- Calculate the sample concentration (C) by using the following formula:

$$X\,[\mu g] / V\,[\mu l] = C\,[\mu g/\mu l] = C\,[mg/ml]$$

X: protein amount of the sample
V: sample volume used in the assay

- Correct for predilution of samples if any by using the following formula:

$$(X\,[\mu g] / V\,[\mu l]) * DF = C\,[\mu g/\mu l] = C\,[mg/ml]$$

X: protein amount of the sample
V: sample volume used in the assay
DF: dilution factor

3. Literature

Lowry O. H., et al. (1951) J. Biol. Chem. **193**, 265 – 275.