UV/VIS Application Note

Biuret Protein Assay

Summary

Biuret assay is commonly used to determine the total protein in biological samples. Peptides with more than two peptide bonds form a purple colored chelate complex with cupric ions in alkaline medium containing sodium potassium tartrate. This assay allows quantification of proteins in the concentration range from 0.5 to approximately 10 mg/mL. Interfering substances are ammonium salts, Tris buffer and the reducing agent dithiothreitol which does enhance the color. Samples are measured at the absorbance maximum of 540 nm to determine the quantity.

Sample and Reagent

Reagents included from
HiPer® Protein Estimation kit – HiMedia®

- Protein Standard, Solution (50 mg/mL)
- Biuret Reagent
- Protein samples 1& 2

Instruments and Accessories

- UV7 Spectrophotometer (ME-30254726)
- CuvetteChanger (ME-30236313)
- Rainin pipettes
  - 100 - 1000µL (ME-17011782)
  - 10 - 100µL (ME-17011781)
  - 500 - 5mL (ME-17011790)
- 10 mL glass test tubes
- Disposable 10 mm pathlength cuvette

Sample preparation

Preparation of working solution

A series of protein standards concentration ranging from 1.0 to 10.0 mg/200 µL is prepared by serial dilution in a 10 mL glass tube.

Solution	Protein (BSA) Std, 50 mg/mL [µL]	Deionized water [µL]	Final conc. [mg/200µL]
S0	0	200	0
*S1	0	200	0
S2	20	180	1
S3	40	160	2
S4	80	120	4
S5	120	80	6
S6	160	40	8
S7	200	0	10
S8	200 of protein sample 1	0	5
S9	200 of protein sample 2	0	2.5

Table1: Preparation of working solutions by serial dilution

*S1 is same solution as S0; here used as zero concentration standard.

Preparation of colorimetric standard S1- S9

10 glass tubes of 10 mL were labelled as S0, S1, S2, S3, S4, S5, S6, S7, S8, and S9. Dilutions of the protein standard concentrations are prepared using the protein BSA and deionized water as mentioned in Table1. 2.0 mL of Biuret reagent is added to each tube and mixed with the help of the pipette. All the glass tube solutions are incubated at room temperature (20 - 25 °C) for 10 min and then transferred to a 10 mm disposable cuvette. Blank measurement is performed using solution S0 before measurement of the standards. Solution S1 is used as zero (0.0) concentration standard, to obtain linear graph from origin. The absorbance of the standards is measured in increasing order of concentration (S2 to S7) followed by the protein sample solutions S8 & S9.

Measurement

Method parameters

Method:	Quant
Path length:	1.0 cm
Automation:	CuvetteChanger*
No. of standards:	7
Concentrations:	0.0, 1.0, 2.0, 4.0, 6.0
	8.0, 10.0 mg/200 µL
No. of calibration curves:	1
Wavelength:	540
Background correction:	None
Fit type 1:	Linear
Calculation R1:	Concentration
Unit:	mg/200 µL

Note: this method is available as a template on the UV5Bio and UV5Nano spectrophotometer. * is not applicable for UV5Nano.

Acceptance criteria	
Coefficient of determination	R2 ≥ 0.95
Test sample S8 (5 mg/200 µL)	4.50 – 5.50 mg/200 µL
Test sample S9 (2.5 mg/200 µL)	2.25 – 2.75 mg/200 µL

Results

Std solution	Concentration Std solution [mg/200 µL]	Absorbance @ 540 nm	Coefficient of determination (R^2)
S1	0.0	0.00020	
S2	1.0	0.13230	
S3	2.0	0.24220	
S4	4.0	0.47362	0.999
S5	6.0	0.69674	
S6	8.0	0.92763	
S7	10.0	1.14874	

From the linear graph of the standard curve, the sample concentrations are determined.

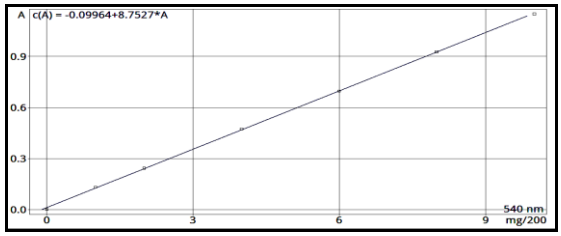

Fig. 1: A540 nm versus concentration of protein standards.

Spl	Expected in mg/200 µL	Recovery in mg/200 µL	Recovery in %	Std Dev	Srel (%)
S8	5.00	5.04	100.8	0.015	0.32
S9	2.50	2.44	97.6	0.007	0.33

Table2: Mean of ten measurements and statistical evaluation

Conclusion

The Biuret assay was successfully performed on the UV7 spectrophotometer. The BSA standard curve was linear up to 10 mg/200 µL. Specified acceptance criteria are met: the coefficient of determination is 0.999 and measurements are found to be repeatable having a Srel of 0.32 % for protein sample 1 and 0.33 % for protein sample 2; with recovery of 100.8 % and 97.6 %.

Reference

HiPer® Protein Estimation Teaching Kit (Quantitative) – HiMedia®

Further information

www.mt.com/uv-vis