Supelco®
Analytical Products

Application Note

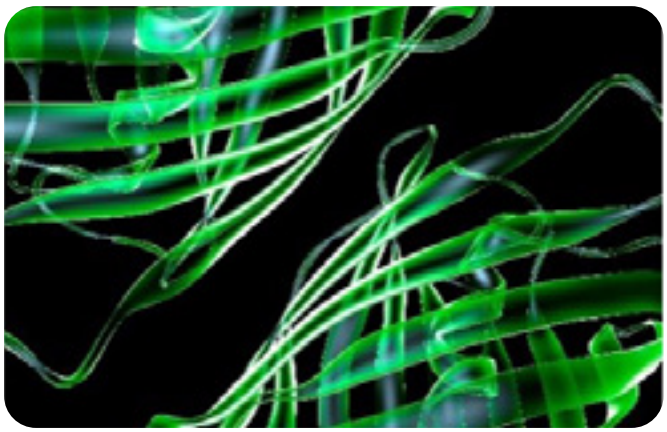
Determination of Protein (Biuret Method)

Photometric Determination with Biuret reagent

Introduction

Proteins are large biomolecules and involved in many physiological processes within organisms. They consist of long chains of amino acids which are connected by peptide bonds.

The quantification of proteins is an important field in bioanalytic. There are different methods to quantify the protein concentration in samples. One method is the Biuret reaction, which is based on the reaction of copper with the above-mentioned peptide bonds to form a blue-violet complex.



Experimental

This Application Note describes the determination of proteins using the Biuret method. The method is preprogrammed on the corresponding Spectroquant® Prove photometers with firmware version 1.5 or above. All reagents required for the measurement are included in the test kit.

More details can be found in the packaging insert of the test kit.

Method

Proteins form a blue-violet complex in alkaline copper sulfate solution containing tartrate (Biuret reagent). The absorbance of the solution is measured at 546 nm. [1-3].

Measuring range

0.5–5.0 g/l protein as bovine serum albumin (method “Protein Biuret LR”, No. 315)

1–10 g/l protein as bovine serum albumin (method “Protein Biuret HR”, No. 316)

Sample material

Aqueous samples or samples after appropriate sample preparation

Reagents, Instruments and Materials:

Cat. No.	Description
Reagents	
110307	Protein Kit (Biuret Method)
Optional, if precipitation step is performed (for colored samples and samples with low concentrations of protein):	
100807	Trichloroacetic acid for analysis EMSURE® ACS
Optional, if user calibration is performed:	
112018	Albumin fraction V (from bovine serum)
Instruments	
For the protein measurement one of the following Spectroquant® photometers is necessary:	
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus
1.73026	Spectroquant® VIS Spectrophotometer Prove 100 plus
Software for data maintenance	
The Spectroquant® Prove Connect to LIMS software package provides an easy way to transfer your data into a preexisting LIMS system. This software can be purchased under:	
Y11086	Prove Connect to LIMS
Materials	
C5291	Rectangular cell 10 mm (polystyrene)*
Pipettes for pipetting volumes of 0.50, 1.0 and 2.0 ml	
Also first generation Prove instruments are compatible and preprogrammed with this method.	

* Strictly avoid the use of quartz cells; the dye adsorbs strongly onto this surface.

Only necessary for turbid solutions:

Filter or centrifuge

Analytical approach

Sample preparation

- The sample solution should be clear. Centrifuge or filter turbid solutions.
- In colored samples and samples with low levels of protein (< 0.5 g/l), the protein should be precipitated from a defined volume with trichloroacetic acid solution and then redissolved in a (smaller) volume of distilled water or a buffer solution.

  Preparation of the trichloroacetic acid (TCA solution): Carefully dissolve 5 g trichloroacetic acid (Cat. No. 100807) in 10 ml redistilled water (50 % (w/v).

  Precipitation Procedure: add 0.2 ml 50 % (w/v) TCA solution per 1 ml of sample solution, mix and centrifuge. Discard the supernatant and dissolve the precipitate in a defined, smaller volume of distilled water.
- Samples with high levels of protein (> 10 g/l) needs to be diluted with distilled water or a buffer solution.

Expected quantity of protein in the sample (g/l)	
< 0.5	TCA precipitation
0.5–5.0	Method “Protein Biuret LR”, No. 315
1–10	Method “Protein Biuret HR”, No. 316
> 10	Dilution

Preparing the measurement solutions

Prepare the measurement sample and the reagent blank depending on the method chosen as follows:

	Measuring range 0.5-5.0 g/l		Measuring range 1-10 g/l	
	Measurement sample	Reagent blank	Measurement sample	Reagent blank
Prepared sample	0.5 ml	-	1.0 ml	-
Distilled water	-	0.5 ml	-	1.0 ml
	• **Pipette** into a plastic test tube.			
Biuret reagent solution	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	• **Mix** thoroughly.			
	• **Leave to stand for 30 min** (reaction time) at room temperature, fill into a 10 mm disposable plastic cell*, then measure with the Spectroquant® Prove spectrophotometer.			

* Strictly avoid the use of quartz cells; the dye adsorbs strongly onto this surface.

Measurement

- Open the method list (<Methods>) and select method No. 315 “Protein Biuret LR” or method No. 316 “Protein Biuret HR”.
- It is recommended to zero the method each new working day. To do this, open the method, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Follow the instructions shown on the display to proceed.
- Subsequently perform the reagent blank (recommended for each new working day). Do this by tapping the <Settings> button and selecting the <REAGENT BLANK> menu item. Fill the 10 mm rectangular plastic cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- If a dilution step has been performed, enter the dilution factor. Do this by tapping the <Settings> button and selecting the <DILUTION> menu item. Enter the dilution factor in the form 1+x.
- Measure the measurement sample. Do this by filling the measurement sample into a 10-mm rectangular plastic cell and inserting the cell into the cell compartment. The measurement starts automatically.
- Read off the result in g/l from the display.

Note

If a precipitation step has been performed, the result of the original sample must be calculated manually by dividing the displayed result with the corresponding enrichment factor.

Data transfer from Prove spectrophotometers

After measurement transfer the values measured on the Prove spectrophotometer using the software “Prove Connect to LIMS”.

Influences of foreign substances

Although few substances interfere with the Biuret method, these happen to be commonly used substances in protein chemistry: e.g. ammonium sulfate, Tris, glycerol and saccharose [5]. If these substances are being used, it is advisable to carry out a TCA precipitation prior to measurement. Lipids can also interfere by causing turbidity; adding up to 3 % sodium deoxycholate can help to prevent this

Calibration

We recommend checking the pre-programmed calibration function of the methods for each new batch of the test kit. For this purpose, an albumin standard solution with a concentration in the middle of the measuring range can be used as sample solution. If significant deviations are detected, the method should be recalibrated. Furthermore, a user-calibration could be useful, if another reference substance than bovine serum albumin should be used.

Preparing the calibration standards

Calibration can be carried out with practically any homogeneous and pure protein. Bovine serum albumin (BSA) is frequently used as a reference substance. To prepare a standard solution, dissolve exactly 1 g BSA (Cat. No. 112018) in 100 ml redistilled water in a volumetric flask. This stock solution (10 g/l) can then be diluted as required:

	Protein Concentration [g/l]					
	0.5	1.0	2.0	3.0	4.0	5.0
Measuring range 0.5–5.0 g/l						
10 g/l BSA Protein stock solution [ml]	0.5	1.0	2.0	3.0	4.0	5.0
distilled water [ml]	9.5	9.0	8.0	7.0	6.0	5.0

	Protein Concentration [g/l]					
	1	2	4	6	8	10
Measuring range 1–10 g/l						
10 g/l BSA Protein stock solution [ml]	1.0	2.0	4.0	6.0	8.0	10
distilled water [ml]	9.0	8.0	6.0	4.0	2.0	-

The standard solutions can be aliquoted and stored for approx. 6 months at -20 °C.

Preparing the measurement solutions for user-defined calibration (re-calibration)

Prepare the standard solutions and the E0 (reagent blank) depending on the method, which should be calibrated, as follows:

	Measuring range 0.5-5.0 g/l		Measuring range 1-10 g/l	
	Standard solution	E0 (Reagent blank)	Standard solution	E0 (Reagent blank)
Prepared sample	0.5 ml	-	1.0 ml	-
Distilled water	-	0.5 ml	-	1.0 ml
	• **Pipette** into a plastic test tube.			
Biuret reagent solution	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	• **Mix** thoroughly.			
	• **Leave to stand for 30 min** (reaction time) at room temperature, fill into a 10 mm disposable plastic cell*, then measure with the Spectroquant® Prove spectrophotometer.			

* Strictly avoid the use of quartz cells; the dye adsorbs strongly onto this surface.

User-defined method calibration

- Open the method list (<Methods>) and select method No. 315 “Protein Biuret LR” or method No. 316 “Protein Biuret HR”.
- It is recommended to zero the method each new working day. To do this, open the method, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Follow the instructions shown on the display to proceed.
- Tap the **<Settings>** button and select the **<RECALIBRATION>** menu item. An input mask pops up.
- Tap three times on **<+>** in the numerical keyboard to create three additional input lines.
- Select the **“Absorbance”** field in the **“E0”** line (selected fields are shown in a blue frame). Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the **“Conc.”** field in the **“1”** line and enter the concentration of **0.5 g/l** for method 315 or **1 g/l** for method 316 for the first calibration solution. Select the “**Absorbance**” field in the “**1**” line. Fill calibration solution 1 into a 10-mm rectangular

plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

- Select the "**Conc**." field in the "**2**" line and enter the concentration of **1.0 g/l** for method 315 or **2 g/l** for method 316 for the second calibration solution. Select the "**Absorbance**" field in the "**2**" line. Fill calibration solution 2 into a 10-mm rectangular plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the "**Conc**." field in the "**3**" line and enter the concentration of **2.0 g/l** for method 315 or **4 g/l** for method 316 for the third calibration solution.Select the "**Absorbance**" field in the "**3**" line. Fill calibration solution 3 into a 10-mm rectangular plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the "**Conc.**" field in the "**4**" line and enter the concentration of **3.0 g/l** for method 315 or **6 g/l** for method 316 for the fourth calibration solution Select the "**Absorbance**" field in the "**4**" line. Fill calibration solution 4 into a 10-mm rectangular plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the "**Conc.**" field in the "**5**" line and enter the concentration of **4.0 g/l** for method 315 or **8 g/l** for method 316 for the fifth calibration solution.Select the "**Absorbance**" field in the "**5**" line. Fill calibration solution 5 into a 10-mm rectangular plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the "**Conc.**" field in the "**6**" line and enter the concentration of **5.0 g/l** for method 315 or **10 g/l** for method 316 for the fifth calibration solution.Select the "**Absorbance**" field in the "**6**" line. Fill calibration solution 6 into a 10-mm rectangular plastic cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Activate the **<U-CAL on>**, **<linear>** and **<Fit E0>** fields. Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.
- Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

Analytical Quality Assurance

The objective of analytical quality assurance (AQA) is to secure correct and precise measurement results. AQA is recommended before each measurement series. To check the measurement system (test reagents, measurement device, and handling) a self-prepared bovine serum albumin standard solution can be used. For details on how to prepare the standards see calibration-preparing the standard solutions. Sample-dependent interferences (matrix effects) can be determined by means of standard addition.

For details on how to perform the AQA check see the instrument-specific manuals.

Conclusion

The Protein (Biuret) test kit is an easy and fast way to analyze the protein concentration in your sample. The measurement can be performed without high instrumental expense. The method is prepogrammed on the Spectroquant® Prove instruments with firmware version 1.5 or above.

For more information

Spectroquant® photometric system see:

SigmaAldrich.com/photometry

References

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2 Doumas, B. T., et al.; Clin. Chem. 27, 1642–1650 und 1651–1654 (1981)

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4 Beisenherz, G., et al.; Z. Naturf. 8b, 555-577 (1953)

5 Thorne, C. J. R.; in: Techniques in Protein and Enzyme Biochemistry (Kornberg, H. L., et al. eds.), Pt. 1, B 104, 1-18, Elsevier-North Holland, Amsterdam (1978)

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