

1.10307.0500

Protein (Biuret Method)

Cat. No. 1.10307.0500

Protein (Biuret Method)
Reagent solution for approx. 250 determinations

Measuring ranges

Range 1: 1 - 10 g/l

Range 2: 0.5 - 5.0 g/l

Method of determination

Proteins form a blue-violet complex in alkaline copper sulfate solution containing tartrate (Biuret reagent). The absorbance of the solution is normally measured at 546 nm. The protein concentration of the sample can be determined by calibrating with a protein standard solution.¹⁻³

Packaging, storage, and stability

Each bottle contains 500 ml Biuret solution and is sufficient for about 250 determinations. The solution is stable for at least 12 months at room temperature. It is stable for at least 2 years if kept in a refrigerator at + 2 to + 8 °C.

Sample preparation

The sample solution should be clear and show no signs of coloring. **Turbid solutions** should be centrifuged or filtered. In **colored samples** and those containing **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 redistilled water.

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

Procedure: To precipitate, 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 redistilled water. This is now the sample solution.

Suspected quantity of protein in the sample (g/l)		
< 0.5		TCA precipitation
0.5 - 5.0		Measuring range 2
1 - 10		Measuring range 1
> 10		Dilution

Preparation of standard solutions

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:

Measuring range 1 (1 - 10 g/l)

Standard solutions (g/l)	1	2	4	6	8	10
Protein stock solution (ml) (10 g/l BSA)	1	2	4	6	8	10
Redistilled water (ml)	9	8	6	4	2	-

Measuring range 2 (0.5 - 5.0 g/l)

Standard solutions (g/l)	0.5	1.0	2.0	3.0	4.0	5.0
Protein stock solution (ml) (10 g/l BSA)	0.5	1.0	2.0	3.0	4.0	5.0
Redistilled water (ml)	9.5	9.0	8.0	7.0	6.0	5.0

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

Carrying out the determination

Measurement should be carried out using a disposable plastic cell (path length 1 cm) at 546 nm. Zero adjustment of the photometer can be carried out against air or water.

Pipetting scheme Measuring range 1 (1 - 10 g/l)	Sample or standard	Reagent blank
Sample solution / standard solution	0.5 ml	-
Redistilled water	-	0.5 ml
Biuret reagent solution	2.0 ml	2.0 ml

Mix thoroughly, incubate for 30 minutes at room temperature and measure absorbance at 546 nm.

Pipetting scheme Measuring range 2 (0.5 - 5.0 g/l)	Sample or standard	Reagent blank
Sample solution / standard solution	1.0 ml	-
Redistilled water	-	1.0 ml
Biuret reagent solution	2.0 ml	2.0 ml

Mix thoroughly, incubate for 30 minutes at room temperature and measure absorbance at 546 nm.

Evaluation

Calculate the differences in absorbance $\Delta E = E_{\text{Standard}} - E_{\text{Blank}}$ and $\Delta E = E_{\text{Sample}} - E_{\text{Blank}}$.

To compile the calibration curve, plot the ΔE values against the corresponding protein standards; the protein concentration of unknown samples can then be determined graphically from the calibration curve or they can be calculated using the slopes of the curves. (The slopes - or "Biuret factors" - of several important proteins have been summarized in tabular form⁴.)

NB: If the sample has been diluted prior to measurement, the result must be multiplied by the appropriate dilution factor *f*.

Interference

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 saccharose⁵. 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.

Ordering information: Reagents

Cat. No.	Designation	Packaging size
110307	Protein (Biuret Method) Reagent solution for approx. 250 determinations	500 ml
100807	Trichloroacetic acid for analysis EMSURE [®] ACS	100 g, 250 g
112018	Albumin, fraction V	25 g, 100 g

There is also another ready-to-use reagent solution for protein determination:

Cat. No.	Designation	Packaging size
110306	Protein (Bradford Method) Reagent solution for approx. 200 determinations	500 ml

Literature references

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2. Dumas, B. T., et al.; Clin. Chem. **27**, 1642–1650 und 1651–1654 (1981)
3. Amtl. Sammlung von Untersuchungsverfahren nach § 35 LMBG: L 06.00–23 (Mai 1986), L 07.00 36 (Mai 1986) und L 08.00–28 (Mai 1986)
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)

