Supelco®

1.10306.0500

Protein (Bradford Method)

Cat No. 1.10306.0500 **Protein (Bradford Method)**
Reagent solution for approx. 200 determinations

Safety precautions

The reagent solution contains phosphoric acid and methanol. Please observe the safety regulations when handling these chemicals. Always wear safety glasses and safety gloves in the laboratory. Should the skin or clothing come into contact with the substances, wash immediately with abundant amounts of water.

Measuring ranges

Range 1: 0.1 - 1.4 mg/ml

Range 2: 0.01 - 0.1 mg/ml (micro)

Method of determination

The method used for the determination of total protein is based on the property of Coomassie® Brilliant Blue G-250 to bind to proteins; in doing so, its absorbance maximum is shifted from 465 nm to 595 nm[1, 8]. The absorption of the sample solution at 595 nm is proportional to the concentration. The dye has an affinity for basic and aromatic amino acids. Its dependence on the type of protein and its possible reaction with various reagents have been intensively investigated (see also the section "Interference")[1-5].

Packaging, storage, and stability

Each bottle contains 500 ml of the **ready-to-use** reagent solution and is sufficient for approximately 200 determinations as stated in the accompanying protocol.
The solution is stable for at least 2 years if kept between +2 and +8 °C in a refrigerator. At room temperature, it is stable for about 12 months.

Sample preparation

Turbid samples should be centrifuged or filtered. If the concentration of protein is high, the sample should be diluted so that measurement can be carried out within the measuring range.

Suspected quantity of protein in the sample (mg/ml)	
0.01 - 0.1	Measuring range 2
0.1 - 1.4	Measuring range 1
> 1.4	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; however, this particular protein is not suitable for calibra-tion of the Bradford assay. In contrast to most other proteins, BSA results in about double the color intensity in standard assays[10] so that the true protein concentration is systematically underestimated. Although less popular, bovine immunoglobulin G is more suitable for this calibration.

TQ prepare a standard solution, dissolve precisely 100 mg of BSA (Cat. No. 112018) in 10 ml of redistilled water in a volumetric flask. This stock solution (10 mg/ml) can be diluted as required:

Measuring range 1 (0.1 - 1.4 mg/ml)

Standard solutions (mg/ml)	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**
Protein stock solution I (ml) (10 mg/ml)	0.20	0.40	0.60	0.80	1.00	1.20	1.40
Redistilled water (ml)	9.80	9.60	9.40	9.20	9.00	8.80	8.60

Measuring range 2 (0.01 - 0.1 mg/ml)

Standard solutions (mg/ml)	**0.01**	**0.02**	**0.04**	**0.06**	**0.08**	**0.1**
Protein stock solution II (ml) (0.1 mg/ml)	0.1	0.2	0.4	0.6	0.8	1.0
Redistilled water (ml)	0.9	0.8	0.6	0.4	0.2	-

Protein stock solution II: Place 1.0 ml of the 1.0 mg/ml standard solution in a 10 ml volumetric flask and make up to the mark with redistilled water.

Carrying out the determination

Measurement should be carried out using a disposable plastic or glass cell (path length 1 cm) at 595 nm. On no account should quartz cells be used; the dye adsorbs strongly onto this surface. Zero adjustment of the photometer can be carried out against air or water.

Pipetting scheme for cell test Measuring range 1 (0.1 - 1.4 mg/ml)	Sample or standard	Reagent blank
Sample solution / standard solution	0.05 ml	-
Redistilled water (or sample buffer)	-	0.05 ml
Bradford reagent solution	2.5 ml	2.5 ml
Mix thoroughly, wait 2 minutes and measure absorbance at 595 nm.		

Pipetting scheme for cell test Measuring range 2 (0.01 - 0.1 mg/ml)	Sample or standard	Reagent blank
Sample solution / standard solution	0.25 ml	-
Redistilled water (or sample buffer)	-	0.25 ml
Bradford reagent solution	2.5 ml	2.5 ml
Mix thoroughly, wait 2 minutes and measure absorbance at 595 nm.		

The test can also be performed using microtitre plates[6], although the volumes and measuring range have to be changed. Also, precision and reproducibility have been shown to be worse than when using a conventional cell test due to the relatively high pipetting error involved.

Pipetting scheme for microtitre plates Measuring range (0.05 - 0.5 mg/ml)	Sample or standard	Reagent blank
Sample solution / standard solution	10 µl	-
Redistilled water (or sample buffer)	-	10 µl
Bradford reagent solution	200 µl	200 µl
Mix thoroughly, wait 2 minutes and measure absorbance at 595 nm.		

Evaluation

To compile a calibration curve, subtract the absorbance of the reagent blank from that of the standard. The difference ΔE can then be plotted against the standard protein concentrations.

$$\Delta E_{Standard} = E_{Standard} - E_{Blank} \text{ or } \Delta E_{Sample} = E_{Sample} - E_{Blank}$$

The calibration curve is rarely linear over the entire concentration range; thus, the protein concentration of an unknown sample should be calculated either graphically or by means of linear regression.
NB: If the sample has been diluted prior to measurement, the result must be multiplied by the appropriate dilution factor f.

Interference

A number of reagents capable of reacting with the dye or the proteins can interfere with the Bradford Protein Assay[8]. The table below shows which reagents and what concentrations can cause interference. Combinations of these reagents can give rise to other reactions that also interfere with the test. Thus, we recommend preparing all blank and standard solutions with the same buffer as used in the preparation of the sample.

Table 1: Concentrations of those reagents that are known to cause interference of the essay:

Inorganic salts	
Ammonium sulfate	> 1 M
KCl	> 1 M
$MgCl_2$	> 1 M
Na azide	> 0.5 %
NaCl	> 5 M
NaSCN	> 3 M

Detergents	
Brij®	> 0.5 %
Desoxycholate	> 0.1 %
SDS	> 0.1 %
Triton® X-100	> 0.1 %
Tween® 20	> 0.5 %

Nucleic acids etc.	
Adensosine	> 1 mM
ATP	> 1mM
DNA	> 1 mg/ml
RNA	> 0.3 mg/ml
rRNA	> 0.25 mg/ml
tRNA	> 0.4 mg/ml
Thymidine	> 1 mM

Organic solvents	
Acetone	-*
Ethanol	-*
Methanol	-*
Phenol	> 5%

Buffers	
Acetate	> 0.5 M
Barbital	-*
BES	> 2.5 M
Boric acid	-*
CHAPS	> 1 %
CHAPSO	> 1 %
Citrate	> 50 mM
Glycine	> 0.1 M
HEPES	> 0.1 M
MES	> 0.7 M
MOPS	> 0.2 M
Phosphate	> 1 M
PIPES	> 0.5 M
Resolytes™	> 0.5 %
Tricine	-*
Tris	> 2 M

Other reagents	
DTT	> 1 M
EDTA	> 0.1 M
Glutathione	-*
Glycerol (99 %)	-*
Guanidinium chloride	-*
Mercaptoethanol	> 1 M

NAD	> 1 mM
Peptides < 3000 D	-*
Peptone	-*
Pyrophosphate	> 0.2 M
Sodium hydroxide	> 0.25 M
Urea	> 6 M

* No interference of the assay has been observed under normal conditions

Ordering information: Reagents

Cat. No.	Designation	Packaging size
110306	Protein (Bradford Method) Reagent solution for approx. 200 determinations	500 ml
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
110307	Protein (Biuret Method) Reagent solution for approx. 250 determinations	500 ml

Literature references

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