MB198_IFU_EN_V2401

NZYBradford reagent

Catalogue number	Presentation
MB19801	500 mL

Description

The NZYBradford reagent for the determination of total protein is based on the property of Brilliant Blue G-250 to bind to proteins. When this dye binds to the basic and aromatic aminoacids of proteins, its absorbance maximum is shifted from 465 nm to 595 nm. This absorbance at 595 nm is proportional to the protein concentration.

Shipping & Storage Conditions

This product can be shipped from Blue Ice to Room temperature. Upon receipt, store NZYBradford reagent at 2°C to 8°C. At room temperature, it is stable for about 12 months.

Components

Each bottle contains 500 mL of the ready-to-use reagent solution and is sufficient for approximately 200 determinations.

COMPONENT	BOTTLE	VOLUME
NZYBradford reagent	1	500 mL

Standard Protocol

Measuring ranges

Range 1: 0.1 – 1.4 mg/mL
Range 2: 0.01 – 0.1 mg/mL

Sample preparation

Turbid samples should be centrifuged or filtered. If the protein concentration is high, the sample should be diluted so that measurements can be carried out within the measuring range. Use the table below as a guide:

SUSPECTED PROTEIN CONCENTRATION (MG/ML)	RANGE
0.01 – 0.1	Range 2
0.1 – 1.4	Range 1
>1.4	Dilution

Preparation of the standard solutions

- Measuring range 1: 0.1 – 1.4 mg/mL

Bovine serum albumin (BSA) is frequently used as a reference protein.

Protein stock solution I: Prepare a BSA standard solution by dissolving precisely 100 mg of BSA Low Endotoxin (MB046) in a final volume of 10 mL of redistilled water. This stock solution (10 mg/mL) can be diluted as required:

STANDARD SOLUTIONS (MG/ML)	PROTEIN STOCK SOLUTION I 10 MG/ML	STANDARD SOLUTIONS (MG/ML)
0.2	0.20	9.80
0.4	0.40	9.60
0.6	0.60	9.40
0.8	0.80	9.20
1.0	1.00	9.00
1.2	1.20	8.80
1.4	1.40	8.60

- Measuring range 2: 0.01 – 0.1 mg/mL

Protein stock solution II: Place 0.1 mL of the 10.0 mg/mL standard solution in a 10 mL volumetric flask and make up to the mark with redistilled water.

STANDARD SOLUTIONS (MG/ML)	PROTEIN STOCK SOLUTION II 0.1 MG/ML	STANDARD SOLUTIONS (MG/ML)
0.01	0.1	0.9
0.02	0.2	0.8
0.04	0.4	0.6
0.06	0.6	0.4
0.08	0.8	0.2
0.10	1.0	-

Procedure

Measurements should be carried out in a spectrophotometer using a disposable plastic or glass cell (path length 1 cm) at 595 nm. Quartz cells are not advised since 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)

MEASURING RANGE 1	SAMPLE OR STANDARD	REAGENT BLANK
Sample solution /standard solution	0.05 mL	-
Distilled water	-	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)

MEASURING RANGE 2	SAMPLE OR STANDARD	REAGENT BLANK
Sample solution /standard solution	0.25 mL	-
Distilled water	-	0.25 mL
Bradford reagent (solution)	2.5 mL	2.5 mL

Mix thoroughly, wait 2 minutes and measure absorbance at 595 nm.

- Pipetting scheme for Pipetting scheme for microtiter plates - Measuring range 2 (0.01 – 0.1 mg/mL)

The test can also be performed using microtiter plates, although the volumes and measuring range must 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.

MEASURING RANGE 2	SAMPLE OR STANDARD	REAGENT BLANK
Sample solution /standard solution	10 µL	-
Distilled water	-	10 µL
Bradford reagent (solution)	200 µL	200 µL

Mix thoroughly, wait 2 minutes and measure absorbance at 595 nm.

Calculation

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.

ΔEStandard = EStandard - EBlank
or
ΔESample = ESample - EBlank

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

Interference

Bradford Protein assay readings may be affected by some reagents present in the sample. 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. To avoid interferences, it is recommended preparing all blank and standard solutions with the same buffer used in the preparation of the sample.

INORGANIC SALTS	Ammonium sulphate	>1 M
	KCl	>1 M
	MgCl2	>1 M
	Na azide	>0.5%
	NaCl	>5 M
	NaSCN	>3 M
	Ammonium sulphate	>1 M
DETERGENTS	Brij	>0.5%
	Desoxycholate	>0.1%
	SDS	>0.1%
	Triton X-100	>0.1%
	Tween 20	>0.5%
NUCLEIC ACIDS	Adenosine	>1 mM
	ATP	>1 mM
	DNA	>1 mg/mL
	RNA	>0.3 mg/mL
	rRNA	>0.25 mg/mL
	tRNA	>0.4 mg/mL
	Thymidine	>1 mM
BUFFERS	Acetate	>0.5 M
	BES	>2.5 M
	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%
	Tris	>2 M

Quality control assays

Functional assay

Quality control for testing Bradford reagent via a Protein Standard Curve (using Bovine Serum Albumin as the standard) at 595 nm involves assessing the linearity, precision, and sensitivity of the assay, validating its reliability for accurately quantifying protein concentrations in samples.