Technical Bulletin

Bradford Reagent

B6916

Storage Temperature 2–8 °C

Product Description

The Bradford Reagent can be used to determine the concentration of proteins in solution. The procedure is based on the formation of a complex between the dye, Brilliant Blue G, and proteins in solution. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm. The amount of absorption is proportional to the protein present.[1] The Bradford Reagent requires no dilution and is suitable for micro, multiwell plate, and standard assays. The linear concentration range is 0.1–1.4 mg/mL of protein, using BSA (bovine serum albumin) as the standard protein.

The Bradford Reagent is compatible with reducing agents. Reducing agents are often used to stabilize proteins in solution. Other protein assay procedures (Lowry and BCA) are not compatible with reducing agents. The Bradford Reagent should be used in place of these protein assays if reducing agents are present. However, the Bradford Reagent is only compatible with low concentrations of detergents (see compatibility chart). If the protein sample to be assayed has detergent(s) present in the buffer, it is suggested to use the BCA protein determination procedure.

Reagent

The product consists of Brilliant Blue G in phosphoric acid and methanol. The 500 mL package is sufficient to perform at least 160 standard 3.1 mL assays.

Reagents and Equipment Required Depending on Assay Format Used

- Spectrophotometer capable of measuring absorbance in the 595 nm region.
- 96-well plates (Cat. No. CLS9017)
- 96-well plate sealing film (Cat. No. Z369667)
- Test tubes, 13 × 100 mm (Cat. No. CLS980013)
- 3 mL Disposable Plastic Cuvettes (Cat. No. C5291)
- 1 mL Disposable Plastic Cuvettes (Cat. No. C5416)
- Protein Standard (BSA) Solution, (2 mg/mL) (Cat. No. P0834)
- Protein Standard (BSA) Solution, (1 mg/mL) (Cat. No. P0914), if lower concentrations of protein are to be measured.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is stored at 2–8 °C. It is stable at 2–8 °C in an unopened container for at least 1 year.

Procedure

The standard 3.1 mL Bradford assay consists of mixing 1 part of the protein sample with 30 parts of the Bradford Reagent. The sample may be a blank, a protein standard, or an unknown sample. The blank consists of buffer with no protein. The protein standard consists of a known concentration of protein, and the unknown sample is the solution to be assayed.

Bradford assays are routinely performed at room temperature. Color development begins immediately. The absorbance at 595 nm is recorded and the protein concentration is determined by comparison to a standard curve.

There are three protocols and it is suggested a new user try the standard 3.1 mL assay protocol first. The micro assay is for samples with low protein concentrations. The 96-well plate assay is for those who wish to perform the Bradford assay in plate format.

Standard 3.1 mL Assay Protocol

(0.1 mL of a 0.1–1.4 mg/mL protein sample is used)

This assay is performed in test tubes. The assay uses 0.1 mL of the protein sample and 3 mL of the Bradford Reagent per tube. It is possible to do an assay directly in a cuvette by adding just 1.5 mL of Bradford Reagent to 0.05 mL of sample.

Note: It is necessary to create a standard curve during each assay, regardless of the format used.

1. Gently mix the Bradford Reagent in the bottle and bring to room temperature.
2. Prepare protein standards of appropriate concentrations in the same buffer as the unknown samples. The standards should be created by serially diluting either the 2 mg/mL or 1 mg/mL BSA protein standard (Table 1). Deionized water may be substituted for the buffer, but any interference due to buffer components will not be compensated for in the protein standards. The protein standards can range from 0.1–1.4 mg/mL. Create a standard assay table similar to Table 1.

Table 1.
Example of Standard Assay Set Up Table

For protein samples with unknown concentrations, it may be necessary to prepare a dilution scheme to ensure the concentration is within the linear range of 0.1–1.4 mg/mL. Tube 6 represents an unknown sample with a 2-fold dilution. Researchers must determine their own dilution schemes based on their estimation of the concentration of each unknown sample.

Tube No.	Sample (mL)	[BSA] protein standard (mg/mL)	Bradford Reagent (mL)
1	0.1	0	3
2	0.1	0.25	3
3	0.1	0.5	3
4	0.1	1.0	3
5	0.1	1.4	3
6	0.1	(unknown)	3

Use either the 1 mg/mL standard (Cat. No. P0914) or the 2 mg/mL standard (Cat. No. P0834) to create the protein standards. Each tube should contain 0.1 mL of a known standard, blank (buffer only), or unknown sample.

3. After adding 3 mL of Bradford Reagent to each tube, they should be vortexed gently for thorough mixing. The total liquid volume in each tube is 3.1 mL.
4. Let the samples incubate at room temperature for 5–45 minutes.
5. Transfer the samples into cuvettes.
6. Measure the absorbance at 595 nm. The protein-dye complex is stable up to 60 minutes. The absorbance of the samples must be recorded before the 60 minute time limit and within 10 minutes of each other.
7. Determine the protein concentration by comparison of the unknown samples to the standard curve prepared using the protein standards (Table 2 and Graph 1).

Table 2.
Example of Assay Data Table

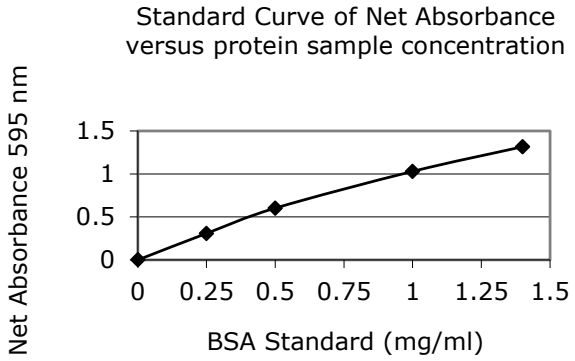
Create a table with the absorbance results obtained from the assay.

Note: The data below should not be used as a replacement of a standard curve. The absorbance of the BSA protein standards (Tubes 1–5) in each assay will differ from those presented here.

Tube No.	A_{595}	Net A_{595}	[Protein] per Assay (mg/mL)	Dilution Factor
1	0.433	0	0	1
2	0.742	0.308	0.25	1
3	1.036	0.602	0.5	1
4	1.463	1.029	1.0	1
5	1.750	1.316	1.4	1
6	1.245	0.811	0.75	2

After obtaining the results, create a standard curve to determine the protein concentration in the unknown sample. Plot the Net Absorbance at 595 nm versus the protein standard concentrations (Tubes 1–5).

Graph 1.
Standard Curve produced from Assay Data



The standard curve indicates the unknown protein sample in test tube 6 (Net A_{595} = 0.811) contains 0.75 mg/mL of protein.

The total concentration of protein present in the original unknown protein solution is calculated as follows:

(mg/mL unknown protein sample) times
(Dilution Factor)
(0.75 mg/mL) × (2) = 1.5 mg/mL of protein

Micro 2 mL Assay Protocol

(1 mL of a 1–10 µg/mL protein sample is used)

The micro assay is used when a large volume (at least 1 mL) of a dilute sample is available for testing. The linear concentration range of this assay is lower than the standard or multiwell plate assays, (1–10 µg of total protein in 1 mL).

1. Gently mix the Bradford Reagent in the bottle and bring to room temperature.
2. Prepare protein standards in buffer ranging from 1–10 µg/mL using a BSA standard or an equivalent protein standard.
3. Add 1 mL of each protein standard to separate tubes. To the tube used as the blank, add 1 mL of buffer.
4. Prepare the unknown sample(s) with an approximate concentration between 1–10 µg/mL. Add 1 mL of each sample to separate tubes.
5. To each tube, add 1 mL of the Bradford Reagent and mix.
6. Let the samples incubate at room temperature for 5–45 minutes.
7. Transfer samples into cuvettes.
8. Measure the absorbance at 595 nm. The protein-dye complex is stable up to 60 minutes. The absorbance of the samples must be recorded before the 60 minute time limit and within 10 minutes of each other.
9. Plot the net absorbance vs. the protein concentration of each standard.
10. Determine the protein concentration of the unknown sample(s) by comparing the Net A_{595} values against the standard curve.

96 Well Plate Assay Protocol

(5 µl of a 0.1–1.4 mg/mL protein sample is used)

This assay is performed in a 96 well plate. With this assay it is possible to quickly assay multiple protein samples, while using a small sample volume (5 µL). It is also possible to automate your protein determination with this multiwell plate assay.

1. Gently mix the Bradford Reagent in the bottle and bring to room temperature.
2. Prepare protein standards in buffer ranging from 0.1–1.4 mg/mL using a BSA standard or an equivalent protein standard.
3. Add 5 µl of the protein standards to separate wells in the 96-well plate. To the blank wells, add 5 µl of buffer.
4. Prepare the unknown sample(s) with an approximate concentration between 0.1–1.4 mg/mL.
5. To each well being used, add 250 µL of the Bradford Reagent and mix on a shaker for ~ 30 seconds.
6. Let the samples incubate at room temperature for 5–45 minutes. Then measure the absorbance at 595 nm. The protein-dye complex is stable up to 60 minutes. The absorbance of the samples must be recorded before the 60 minute time limit and within 10 minutes of each other.
7. Plot the net absorbance vs. the protein concentration of each standard.
8. Determine the protein concentration of the unknown sample(s) by comparing the Net A_{595} values against the standard curve.

Compatibility Chart

The concentration listed below is the maximum amount of material, which can be present in the protein sample without causing interference in the standard or 96-well plate assay protocols. Since a larger volume of protein sample is used in the micro assay, the micro assay is compatible with amounts lower than those listed below.

Incompatible Substances	Amount Compatible
Buffer Systems	
ACES, pH 7.8	100 mM
N-Acetylglucosamine in PBS, pH 7.2	100 mM
Bicine, pH 8.4	100 mM
Bis-Tris, pH 6.5	100 mM
Calcium chloride in TBS, pH 7.2	10 mM
CelLytic™ B Reagent	1:10 dilution
CHES, pH 9.0	100 mM
Cobalt chloride in TBS, pH 7.2	10 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
Glycine	100 mM
HEPES, pH 7.5	100 mM
Imidazole, pH 7.0	200 mM
MES (0.1 M), NaCl (0.9%), pH 4.7	undiluted
MES, pH 6.1	100 mM
MOPS, pH 7.2	100 mM
Nickel chloride in TBS, pH 7.2	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	undiluted
PIPES, pH 6.8	100 mM
Sodium acetate, pH 4.8	180 mM
Sodium bicarbonate	0.1 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium Citrate (0.6 M), MOPS (0.1 M), pH 7.5	undiluted
Sodium phosphate	0.1 M
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	undiluted
Tricine, pH 8.0	100 mM
Triethanolamine, pH 7.8	100 mM
Tris	2.0 M

Incompatible Substances	Amount Compatible
Tris (25 mM), Glycine (192 mM), pH 8.0	undiluted
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3	1:2 dilution
Zinc chloride in TBS, pH 7.2	10 mM
Buffer Additives	
Ammonium sulfate	1.0 M
Aprotinin	10 mg/L
Asparagine	10 mM
Cesium bicarbonate	0.1 M
Glucose	1.0 M
Glycerol	10%
Guanidine HCl	3.5 M
Hydrochloric Acid	0.1 M
Imidazole, pH 7.0	200 mM
Leupeptin	10 mg/L
Phenol Red	0.5 mg/mL
PMSF	1 mM
Sodium azide	0.5%
Sodium chloride	5.0 M
Sodium Hydroxide	0.1 M
Sodium orthovanadate in PBS, 1 mM	1 mM
Thimerosal	0.01%
Sucrose	10%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea 3.0 M	3.0 M
Detergents	
BRIJ® 35	0.125%
BRIJ® 52	0.031%
CHAPS	5%
CHAPSO	5%
Deoxycholic acid	0.050%
Nonidet P-40 (IGEPAL® CA-630)	0.5%
SB3-14	0.125%
Octyl β-glucoside	0.5%
Octyl β-thioglucopyranoside	3%
SDS	0.125%
SPAN™ 20	0.5%
TRITON™ X-100	<0.1%
TRITON™ X-114	0.125%
TRITON™ X-305	0.5%
TRITON™ X-405	0.5%
TWEEN® 20	0.062%
TWEEN® 60	0.1%
TWEEN® 80	0.062%
Chelating agents	
EDTA 100 mM	100 mM
EGTA 2 mM	2 mM
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Reducing & Thiol Containing Agents	
2-Mercaptoethanol	1.0 M
Ascorbic acid	50 mM
Cysteine	10 mM
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	5 mM
Potassium thiocyanate	3.0 M
Solvents	
Acetone	10%
Acetonitrile	10%
DMF	10%
DMSO	10%
Ethanol	10%
Methanol	10%

Note: This is not a complete compatibility chart. There are many substances that can affect different proteins in different ways. One may assay the protein of interest in deionized water alone, then in buffer with possible interfering substances. Comparison of the readings will indicate if an interference exists. Refer to the literature for additional information on interfering substances.[1-7]

Reagents that change the pH of the assay or contains high levels of detergents will interfere with the Bradford assay.

Troubleshooting guide

The absorbance of the unknown protein sample is too high.

1. Make sure there are no interfering substances present in the buffer. Test this by diluting the standard protein samples in the same buffer as the unknown samples.
2. The concentration of protein in the unknown sample may be too high. Dilute the unknown sample(s), accordingly.
3. If the volume of the protein sample is small, try using the micro assay procedure or use the QuantiPro™ BCA assay kit (Cat. No. QPBCA).

The protein sample contains incompatible substance(s).

If the concentration of the protein sample is high enough, dilute the sample to reduce the level of the interfering substance.

Technical Tips

1. Make sure the glassware being used has been cleaned well.
2. Make sure that the Bradford Reagent is at room temperature when performing the assay. Also make sure that the Bradford Reagent is gently mixed.
3. Consider a different protein assay procedure. If certain incompatible substance(s) cannot be removed from the assay, consider the use of the BCA Assay (Cat. No. BCA1).
4. If levels of your protein are too low, try using the QuantiPro™ BCA Kit (Cat. No. QPBCA).

References

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