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Pierce™ Bradford Plus Protein Assay Reagent

Catalog Number 23238

Pub. No. MAN0029520 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ Pierce™ Bradford Plus Protein Assay Reagentis a quick and ready-to-use Coomassie-binding, colorimetric method for total protein quantitation. This modification of the well-known Bradford method greatly reduces the tendency of Coomassie reagents to give nonlinear response curves. The formulation substantially improves linearity for a defined range of protein concentration. In addition, the Pierce™ Bradford Plus Protein Assay Reagent results in significantly less protein-to-protein variation than is observed with other Bradford-type Coomassie formulations.

When Coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue.

Performing the assay in either test tube or microplate format is simple: Combine a small amount of protein sample with the assay reagent, mix well, then measure the absorbance at 595 nm.

Note: Protein concentrations should be estimated by reference to absorbance measurements obtained for a series of standard protein dilutions. These are assayed alongside the unknown samples. Excellent protein standards, including Thermo Scientific™ Albumin (BSA) Standard Ampules (Cat. No. 23209) and Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible 0.125-2 mg/mL (Cat. No. A55863) are available. See "Related Thermo Scientific™ products" on page 5.

Contents and storage

The Pierce™ Bradford Plus Protein Assay Reagent, Cat. No. 23238, contains sufficient reagent for 200 test tube assays or 1,000 microplate assays.

Contents	Storage[1]
Pierce™ Bradford Plus Protein Assay Reagent, 300 mL containing Coomassie G-250 dye, methanol, phosphoric acid, and solubilizing agents in water.	4°C

[1] Product shipped at ambient temperature.

Note: Discard any reagent that shows discoloration or evidence of microbial contamination.

Workflow

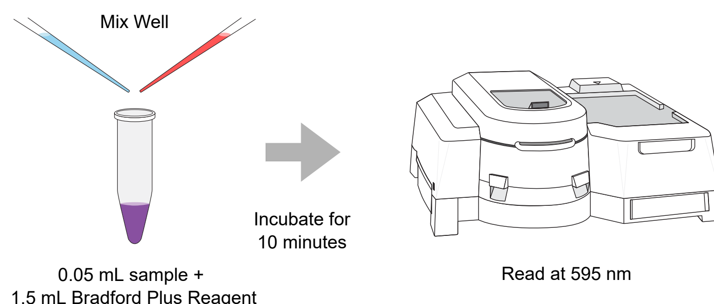

Figure 1 Procedure Summary

Preparation of standards and assay reagent

Preparing the BSA standards

The following example uses the Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, Cat. No. A55863.

Each tube of the tubestrip contains 1 mL of corresponding BSA concentration in a format compatible with a multichannel pipette. One tube is supplied empty allowing the addition of a blank solution.

1. When ready to use, carefully remove foil from the tubestrip.
2. After foil is removed, the provided strip cap will be used to reseal the tubes. To keep the strip cap oriented, there is a small hole at one end of the cap strip and should be aligned with the 2 mg/mL standard. The tube strip has a single label on the tube containing the 2 mg/mL solution.
3. Push the cap strip firmly into the tubestrip. Ensure that each of the 8 caps fit snugly in each of the 8 tubes. Store at 4°C.

Preparing the protein standards

The following example uses the Albumin Standard Ampules, Cat. No. 23209.

Use Table 1 and Table 2 as guides in preparing fresh sets of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule (2.0 mg/mL) into several clean vials, preferably in the same diluent as the sample(s). Each 1 mL ampule of Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Tables 1 and 2. There will be sufficient volume for 3 replications of each diluted standard. If using a different protein standard, prepare dilutions spanning a similar concentration range as described in Tables 1 and 2.

Dilution scheme for standard test tube and microplate protocols

Table 1 Dilution scheme for standard test tube and microplate protocols (Working range = 100–1,500 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µL of Stock	2,000 µg/mL
B	125 µL	375 µL of Stock	1,500 µg/mL
C	325 µL	325 µL of Stock	1,000 µg/mL
D	175 µL	175 µL of vial B dilution	750 µg/mL
E	325 µL	325 µL of vial C dilution	500 µg/mL
F	325 µL	325 µL of vial E dilution	250 µg/mL
G	325 µL	325 µL of vial F dilution	125 µg/mL
H	400 µL	100 µL of vial G dilution	25 µg/mL
I	400 µL	0	0 µg/mL = Blank

Table 2 Dilution scheme for standard test tube and microplate protocols (Working range = 1–25 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	2,370 µL	30 µL of Stock	25 µg/mL
B	4,950 µL	50 µL of Stock	20 µg/mL
C	3,970 µL	30 µL of Stock	15 µg/mL
D	2,500 µL	2,500 µL of vial B dilution	10 µg/mL
E	2,000 µL	2,000 µL of vial D dilution	5 µg/mL
F	1,500 µL	1,500 µL of vial E dilution	2.5 µg/mL
G	5,000 µL	0	0 µg/mL = Blank

Mixing, then equilibrating the Bradford Plus Reagent

1. Mix the Bradford Plus Reagent solution immediately before use by gently inverting the bottle several times. Do not shake the bottle to mix the solution.

   Note: Bradford Plus Reagent contains additives that retard the formation of the dye-dye and dye-protein aggregates that tend to form in all Coomassie-based reagents. If left undisturbed, the aggregates will become large enough to be visible. When left overnight

in a clear glass tube, the reagent forms dye-dye aggregates that are visible as a dark precipitate in the bottom of the tube with nearly colorless liquid above. While dye-dye aggregates can form over several hours in stored reagent, dye-protein aggregates form more quickly. Gentle mixing completely disperses the dye-dye aggregates. It is good practice to mix the Bradford Plus Reagent before pipetting and to mix each tube or plate immediately before measuring absorbances.

2. Remove the amount of reagent needed and equilibrate it to room temperature before use.

Test tube procedures

Standard test tube protocol (working range = 100-1500 µg/mL)

The linear working range with BSA = 125-1,000 µg/mL. The linear working range with IgG = 125-1,500 µg/mL.

1. Pipette 0.05 mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5 mL of the Bradford Plus Reagent to each tube, then mix well.
3. Incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water.
5. Measure the absorbance of all the samples.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Micro test tube protocol (working range = 1-25 µg/mL)

1. Pipette 1.0 mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.0 mL of the Bradford Plus Reagent to each tube, then mix well.
3. Incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water.
5. Measure the absorbance of all the samples.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate procedures

When compared to the standard test tube protocol, 595 nm measurements obtained with the microplate protocols are lower because the light path used is shorter. This may increase the minimum detection level of the assay. If higher 595 nm measurements are required, use 7-10 µL of standard or sample and 250 µL of Bradford reagent per well.

Standard microplate protocol (working range = 100-1500 µg/mL)

1. Pipette 10 µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 300 µL of the Bradford Plus Reagent to each well, then mix with plate shaker for 30 seconds.
3. Remove plate from shaker.
4. Incubate plate for 10 minutes at room temperature (RT).
5. Measure the absorbance at or near 595 nm with a plate reader.

6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   When compared to the Standard Test Tube Protocol, 595 nm measurements obtained with the Microplate Protocols are lower, because the light path is shorter. This may increase the assay's minimum detection level. If higher 595 nm measurements are required, use 15 µL of standard or sample and 300 µL of Bradford Plus Reagent per well.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Micro microplate protocol

Working range = 1-25 µg/mL

1. Pipette 150 µL of each standard or unknown sample into the appropriate microplate wells.

2. Add 150 µL of the Bradford Plus Reagent to each well, then mix with plate shaker for 30 seconds.

3. Remove plate from shaker.

4. Incubate plate for 10 minutes at room temperature (RT).

5. Measure the absorbance at or near 595 nm on a plate reader.

6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average blank corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Using the standard curve, determine the protein concentration estimate for each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Observation	Possible cause	Recommended action
Standards and samples yield lower values than expected, while absorbance of blank is acceptable	Reagent was stored improperly.	Refrigerate stored reagent.
	The reagent was too cold.	Allow reagent to warm to RT.
	The absorbance was measured at an incorrect wavelength.	Measure absorbance near 595 nm.
Samples yield lower values than expected, while absorbances of blank and standards are acceptable	The molecular weight of the sample protein (peptide) was too low (e.g., less than 3000 Da).	Use the Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit (Cat. No. A55860) or the Pierce™ BCA Protein Assay Kit (Cat. No. 23227).
A precipitate forms in all tubes	Sample contained a surfactant (detergent).	Dialyze or dilute the sample.
		Remove interfering substances form the samples using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215 (see "Interfering substances" on page 5).
All tubes (including blanks) are dark blue	The sample volume was too large or a strong alkaline buffer was present, raising the pH of the formulation.	Dialyze or dilute the sample.
		Remove interfering substances form the samples using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215 (see "Interfering substances" on page 5).
Need to read absorbances at a different wavelength	Spectrophotometer or plate reader did not have a 595 nm filter.	The color may be read at any wavelength between 575 nm and 615 nm. However, the slope of the standard curve and overall assay sensitivity will be reduced.

Related Thermo Scientific™ products

Contents	Cat. No.
Pierce™ 96-Well Polystyrene Plates, Corner Notch, 100/pkg	15041
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL	A55863
Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set	23208
Pierce™ Bovine Gamma Globulin Standard Ampules, 2 mg/mL, 10 x 1 mL	23212
Pierce™ Bovine Gamma Globulin Standard Pre-Diluted Set, 7 x 3.5 mL	23213
Pierce™ BCA Protein Assay Kit, working range of 20-2000 µg/mL	23227
Micro BCA™ Protein Assay Kit, working range 0.5-20 µg/mL	23235
Compat-Able™ Protein Assay Preparation Reagent Set, sufficient reagents to pre-treat 500 samples to remove interfering substances prior to total protein quantitation	23215
Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit	A55860
Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible	A55866

Additional information

Interfering substances

Certain substances are known to interfere with Coomassie-based protein assays including most ionic and nonionic detergents, which reduce color development and can cause precipitation of the assay reagent. Other substances interfere to a lesser extent. These have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the Standard Test Tube Protocol are listed in “Assay compatibility for various substances” on page 6. Substances were compatible in the Standard Test Tube Protocol if the error in protein concentration estimation (of BSA at 1000 µg/mL) caused by the presence of the substance in the sample was less than or equal to 10%. The blank-corrected 595 nm absorbance measurements (for the 1000 µg/mL BSA standard + substance) were compared to the net 595 nm absorbances of the 1000 µg/mL BSA standard prepared in 0.9% saline.

The effects of interfering substances may be overcome by several methods:

- Remove the interfering substance by dialysis or desalting.
- Dilute the sample until the substance no longer interferes.
- Precipitate proteins with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered should be discarded and the protein pellet solubilized in a small amount of ultrapure water or in the Bradford Plus Reagent.

  Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).
- Remove the interfering substance using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215.

Assay compatibility for various substances

Note: For a more extensive list of substances, download Tech Tip: Protein Assay Compatibility Table from our website . This tech tip includes compatible substances for many of our protein assays and enables easy comparisons.

Table 3 Compatible substance concentrations in the Bradford Plus Assay

Salts/Buffers	Compatible Concentration
ACES, pH 7.8	100 mM
Ammonium sulfate	1 M
Asparagine	10 mM
Bicine, pH 8.4	100 mM
Bis-Tris, pH 6.5	100 mM
Borate (50 mM), pH 8.5 (Cat. No. 28384)	undiluted
B-PER™ Reagent (Cat. No. 78248)	1:2 dilution[1]
Calcium chloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 m), pH 9.4 (Cat. No. 28382)	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (Cat. No. 28386)	undiluted
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (Cat. No. 28388)	undiluted
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
Guanidine•HCl	3.5 M
HEPES, pH 7.5	100 mM
Imidazole,pH 7.0	200 mM
MES, pH 6.1	100 mM
MES (0.1M), NaCl (0.9%), pH 4.7 (Cat. No. 28390)	undiluted
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4 (Cat. No. 28374)	undiluted
Nickel chloride in TBS, pH 7.2	10 mM
PBS: Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (Cat. No. 28372)	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer: 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1:10 dilution[1]
Sodium acetate, pH 4.8	180 mM
Sodium azide	0.5%
Sodium bicarbonate	100 mM
Sodium chloride	5 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium phosphate	100 mM
Tricine,pH 8.0	100 mM
Triethanolamine,pH 7.8	100 mM

Salts/Buffers	Compatible Concentration
Tris	2 M
TBS:Tris (25 mM), NaCl (0.15 M), pH 7.6 (Cat. No. 28376)	undiluted
Tris (25 mM), Glycine (192 mM), pH 8.0 (Cat. No. 28380)	undiluted
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 28378	1:2 dilution[1]
Zinc chloride in TBS, pH 7.2	10 mM

[1] Diluted with ultrapure water

Detergents	Compatible Concentration
Brij™-35	0.125%
Brij™-56, Brij™-58	0.031%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.05%
Lubrol™ PX	0.125%
Octyl β-glucoside	0.5%
Octyl β-thioglucopyranoside	3%
Nonidet P-40 (NP-40)	0.5%
SDS	0.125%
Span™-20	0.5%
Triton-X™-100, Triton-X™-114	0.125%
Triton-X™-305, Triton-X™-405	0.5%
Tween™-20, Tween™-80	0.062%
Tween™-60	0.1%
Zwittergent™ 3-14	0.025%

Chelating Agents	Compatible Concentration
EDTA	100 mM
EGTA	2 mM
Sodium citrate	200 mM

Reducing and Thiol-containing Agents	Compatible Concentration
N-acetylglucosamine in PBS, pH 7.2	100 mM
Ascorbic acid	50 mM
Cysteine	10 mM
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	5 mM
Glucose	1 M
Melibiose	100 mM
2-Mercaptoethanol	1 M
Potassium thiocyanate	3 M
Thimerosal	0.01%

Misc. Reagents and Solvents	Compatible Concentration
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
Ethanol	10%
Glycerol (fresh)	10%
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Methanol	10%
Phenol Red	0.5 mg/mL
PMSF	1 mM
Sodium Hydroxide	100 mM
Sodium vanadate (sodium salt), in PBS, pH 7.2	1 mM
Sucrose	10%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea	3 M

Response characteristics for different proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins. These differences relate to amino acid sequence, isoelectric point, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the color response of the protein. Most protein assay methods utilize BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined. Albumin Standard Ampules (BSA) (Cat. No. 23209) provide a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Bradford Plus Assay (see Figure 2). For greatest accuracy, the standard curve should be prepared from a pure sample of the target protein to be measured.

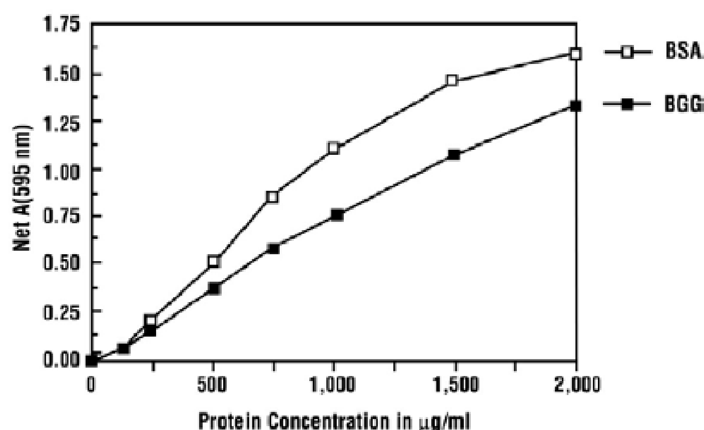

Figure 2 Typical color response curves for BSA and BGG using the standard test tube protocol of the Bradford assay

Table 4 shows typical protein-to-protein variation in color response. All proteins were tested at a concentration of 1000 µg/mL using the Standard Test Tube Protocol. The average net color response for BSA was normalized to 1.00 and the average net color response of the other proteins is expressed as a ratio to the response of BSA. The protein-to-protein variation observed with the Bradford Plus Reagent is significantly less than that seen with other Bradford-type coomassie dye formulations.

Table 4 Protein-to-Protein Variation. Absorbance ratios (595nm) for proteins relative to BSA using the Standard Test Tube Protocol in the Pierce™ Bradford Plus Protein Assay Kit

Protein Tested	Ratio[1]
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.74
α-Chymotrypsinogen, bovine	0.52
Cytochrome C, horse heart	1.03
Gamma globulin, bovine	0.58
IgG, bovine	0.63
IgG, human	0.66
IgG, mouse	0.62
IgG, rabbit	0.43
IgG, sheep	0.57
Insulin, bovine pancreas	0.67
Myoglobin, horse heart	1.15
Ovalbumin	0.68
Transferrin, human	0.90
Average ratio	**0.73**
Standard Deviation	**0.21**
Coefficient of Variation	**28.8%**

[1] Ratio = (Avg "test" net Abs.)/Avg. BSA net ABS)

Measuring absorbances at wavelengths other than 595 nm

If a photometer or plate reader is not available with a 595 nm filter, the blue color may be measured at any wavelength between 570 nm and 610 nm. The maximum sensitivity of the assay occurs when the absorbance of the dye-protein complex is measured at 595 nm. Measuring the absorbance at any wavelength other than 595nm will result in a lower slope for the standard curve and may increase the minimum detection level for the protocol.

Effect of temperature on 595 nm absorbance

Absorbance measurements at 595 nm obtained with the Bradford Plus Reagent are dependent on the temperature of the reagent to some extent. As the reagent temperature increases to room temperature, the 595 nm measurements will increase. Therefore, it is important that the Bradford Plus Reagent remain at a constant temperature (i.e., RT) during the assay.

Cleaning and re-using glassware

Care must be exercised when cleaning glassware that will be used again for protein assays. Thorough cleaning often requires the use of a detergent (such as PCC-54™ Detergent Concentrate, Cat. No. 72288), which must be completely removed in the final rinse. The coomassie dye will stain glass or quartz cuvettes. Disposable polystyrene cuvettes are a convenient alternative.

References

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248-54.

Compton, S.J. and Jones, C.J. (1985). Mechanism of dye response and interference in the Bradford protein assay. *Anal Biochem* **151**:369-74.

Davies, E.M. (1988). Protein assays: A review of common techniques. *Amer Biotech Lab* **July** 28-37.

Sedmak, J.J. and Grossberg, S.E. (1977). A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue brilliant blue G-250. *Anal Biochem* **79**:544-52.

Tal, M., Silberstein, A. and Nusser, E. (1980). Why does Coomassie brilliant blue R interact differently with different proteins? *J Biol Chem* **260**:9976-80.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	17 July 2023	New document for Bradford Plus Protein Assay Reagent.

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