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Figure 1. Coomassie brilliant blue G-250, the binding dye for the Bradford Method Color reaction of protein and Bradford reagent The Bradford assay, a colorimetric protein assay, is based on an absorbance shift of the dye Coomassie brilliant blue G-250. The Coomassie brilliant blue G-250 dye exists in three forms: anionic (blue), neutral (green), and cationic (red).[3] Under acidic conditions, the dye is red; as it loses acidity, the red form of the dye is converted into its blue form, binding to the protein being assayed. If there is no protein to bind, then the solution will remain brown. The dye forms a strong, noncovalent complex with the protein's carboxyl group by van der Waals force and amino group through electrostatic interactions.[1] During the formation of this complex, the red form of Coomassie dye first donates its free electron to the ionizable groups on the protein, which causes a disruption of the protein's native state, consequently exposing its hydrophobic pockets. These pockets in the protein's tertiary structure bind non-covalently to the non-polar region of the dye via the first bond interaction (van der Waals forces) which position the positive amine groups in proximity with the negative charge of the dye. The bond is further strengthened by the second bond interaction between the two, the ionic interaction. When the dye binds to the protein, it causes a shift from 465 nm to 595 nm, which is why the absorbance readings are taken at 595 nm.[4] The cationic (unbound) form is green / red and has an absorption spectrum maximum historically held to be at 465 nm. The anionic bound form of the dye which is held together by hydrophobic and ionic interactions, has an absorption spectrum maximum historically held to be at 595 nm.[5] The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the amount (concentration) of protein present in the sample.[6] Unlike other protein assays, the Bradford protein assay is less susceptible to interference by various chemical compounds such as sodium, potassium or even carbohydrates like sucrose, that may be present in protein samples.[2] An exception of note is elevated concentrations of detergent. Sodium dodecyl sulfate (SDS), a common detergent, may be found in protein extracts because it is used to lyse cells by disrupting the membrane lipid bilayer and to denature proteins for SDS-PAGE. While other detergents interfere with the assay at high concentration, the interference caused by SDS is of two different modes, and each occurs at a different concentration. When SDS concentrations are below critical micelle concentration (known as CMC, 0.00333%W/V to 0.0667%) in a Coomassie dye solution, the detergent tends to bind strongly with the protein, inhibiting the protein binding sites for the dye reagent. This can cause underestimations of protein concentration in solution. When SDS concentrations are above CMC, the detergent associates strongly with the green form of the Coomassie dye, causing the equilibrium to shift, thereby producing more of the blue form. This causes an increase in the absorbance at 595 nm independent of protein presence.[6] Other interference may come from the buffer used when preparing the protein sample. A high concentration of buffer will cause an overestimated protein concentration due to depletion of free protons from the solution by conjugate base from the buffer. This will not be a problem if a low concentration of protein (subsequently the buffer) is used.[6] In order to measure the absorbance of a colorless compound a Bradford assay must be performed. Some colorless compounds such as proteins can be quantified at an Optical Density of 280 nm due to the presence of aromatic rings such as tryptophan, tyrosine and phenylalanine but if none of these amino acids are present then the absorption cannot be measured at 280 nm.[7] Many protein-containing solutions have the highest absorption at 280 nm in the spectrophotometer, the UV range. This requires spectrophotometers capable of measuring in the UV range, which many cannot. Additionally, the absorption maxima at 280 nm requires that proteins contain aromatic amino acids such as tyrosine (Y), phenylalanine (F) and/or tryptophan (W). Not all proteins contain these amino acids, a fact which will skew the concentration measurements. If nucleic acids are present in the sample, they would also absorb light at 280 nm, skewing the results further. By using the Bradford protein assay, one can avoid all of these complications by simply mixing the protein samples with the Coomassie brilliant blue G-250 dye (Bradford reagent) and measuring their absorbances at 595 nm, which is in the visible range[8] and may be accurately measured by the use of a mobile smartphone camera.[9] The procedure for Bradford protein assay is very easy and simple to follow. It is done in one step where the Bradford reagent is added to a test tube along with the sample. After mixing well, the mixture almost immediately changes to a blue color. When the dye binds to the proteins through a process that takes about 2 minutes, a change in the absorption maximum of the dye from 465 nm to 595 nm in acidic solutions occurs.[2] Additionally, protein binding triggers a metachromatic reaction, evidenced by the emergence of a species that absorbs light around 595 nm, indicative of the unprotonated form[10] This dye creates strong noncovalent bonds with the proteins, via electrostatic interactions with the amino and carboxyl groups, as well as Van Der Waals interactions. Only the molecules that bind to the proteins in solution exhibit this change in absorption, which eliminates the concern that unbound molecules of the dye might contribute to the experimentally obtained absorption reading. This process is more beneficial since it is less pricey than other methods, easy to use, and has high sensitivity of the dye for protein.[11] After 5 minutes of incubation, the absorbance can be read at 595 nm using a spectrophotometer or a mobile smartphone camera (RGBradford method).[9] This assay is one of the fastest assays performed on proteins.[12] The total time it takes to set up and complete the assay is under 30 minutes.[13] The entire experiment is done at room temperature. The Bradford protein assay can measure protein quantities as little as 1 to 20 µg.[14] It is an extremely sensitive technique. The dye reagent is a stable ready to use product prepared in phosphoric acid. It can remain at room temperature for up to 2 weeks before it starts to degrade. Protein samples usually contain salts, solvents, buffers, preservatives, reducing agents and metal chelating agents. These molecules are frequently used for solubilizing and stabilizing proteins. Other protein assay like BCA and Lowry are ineffective because molecules like reducing agents interfere with the assay.[15] Using Bradford can be advantageous against these molecules because they are compatible to each other and will not interfere.[16] The linear graph acquired from the assay (absorbance versus protein concentration in µg/mL) can be easily extrapolated to determine the concentration of proteins by using the slope of the line. It is a sensitive technique. It is also very simple: measuring the OD at 595 nm after 5 minutes of incubation. This method can also make use of a Vis spectrophotometer[17] or a mobile smartphone camera (RGBradford method).[9] The Bradford assay is linear over a short range, typically from 0 µg/mL to 2000 µg/mL, often making dilutions of a sample necessary before analysis. In making these dilutions, error in one dilution is compounded in further dilutions resulting in a linear relationship that may not always be accurate. Basic conditions and detergents, such as SDS, can interfere with the dye's ability to bind to the protein through its side chains.[12] The reagents in this method tend to stain the test tubes. Same test tubes cannot be used since the stain would affect the absorbance reading. This method is also time sensitive. When more than one solution is tested, it is important to make sure every sample is incubated for the same amount of time for accurate comparison.[18] A limiting factor in using Coomassie-based protein determination dyes stems from the significant variation in color yield observed across different proteins[19] This limiting factor is notably evident in collagen-rich protein samples, like pancreatic extracts, where both the Lowry and Bradford methods tend to underestimate protein content. It is also inhibited by the presence of detergents, although this problem can be alleviated by the addition of cyclodextrins to the assay mixture.[20] Much of the non-linearity stems from the equilibrium between two different forms of the dye which is perturbed by adding the protein. The Bradford assay linearizes by measuring the ratio of the absorbances, 595 over 450 nm. This modified Bradford assay is approximately 10 times more sensitive than the conventional one.[21] The Coomassie Blue G250 dye used to bind to the proteins in the original Bradford method readily binds to arginine and lysine groups of proteins. This is a disadvantage because the preference of the dye to bind to these amino acids can result in a varied response of the assay between different proteins. Changes to the original method, such as increasing the pH by adding NaOH or adding more dye have been made to correct this variation. Although these modifications result in a less sensitive assay, a modified method becomes sensitive to detergents that can interfere with sample.[22] New modifications for an improved Bradford Protein Assay have been underway that specifically focuses on enhancing detection accuracy for collagen proteins. One notable modification involves incorporating small amounts, approximately .0035%, of sodium dodecyl sulfate (SDS). This inclusion of SDS has been shown to result in a fourfold increase in color response for three key collagen proteins—Collagen types I, III, and IV—while simultaneously decreasing the absorbance of non-collagen proteins.[19] This simple modification in the preparation of the reagent resulted in Bradford Assays to produce similar response curves for both collagen and non-collagen proteins, expanding the use of Bradford Assays in samples containing high collagen proteins. Lyophilized bovine plasma gamma globulin Coomassie brilliant blue 1 0.15 M NaCl Spectrophotometer and cuvettes or a mobile smartphone camera (RGBradford method).[9] Micropipettes Prepare a series of standards diluted with 0.15 M NaCl to final concentrations of 0 (blank = No protein), 250, 500, 750 and 1500 µg/mL. Also prepare serial dilutions of the unknown sample to be measured. Add 100 µL of each of the above to a separate test tube (or spectrophotometer tube if using a Spectronic 20). Add 5.0 mL of Coomassie Blue to each tube and mix by vortex, or inversion. Adjust the spectrophotometer to a wavelength of 595 nm, using the tube which contains no protein (blank). Wait 5 minutes and read each of the standards and each of the samples at 595 nm wavelength. Plot the absorbance of the standards vs. their concentration. Compute the extinction coefficient and calculate the concentrations of the unknown samples. Prepare standard concentrations of protein of 1, 5, 7.5 and 10 µg/mL. Prepare a blank of NaCl only. Prepare a series of sample dilutions. Add 100 µL of each of the above to separate tubes (use microcentrifuge tubes) and add 1.0 mL of Coomassie Blue to each tube. Turn on and adjust a spectrophotometer to a wavelength of 595 nm, and blank the spectrophotometer using 1.5 mL cuvettes or use a mobile smartphone camera (RGBradford method).[9] Wait 2 minutes and read the absorbance of each standard and sample at 595 nm. Plot the absorbance of the standards vs. their concentration. Compute the extinction coefficient and calculate the concentrations of the unknown samples. In summary, in order to find a standard curve, one must use varying concentrations of BSA (Bovine Serum Albumin)[2] in order to create a standard curve with concentration plotted on the x-axis and absorbance plotted on the y-axis. Only a narrow concentration of BSA is used (2-10 ug/mL) in order to create an accurate standard curve.[23] Using a broad range of protein concentration will make it harder to determine the concentration of the unknown protein. This standard curve is then used to determine the concentration of the unknown protein. The following elaborates on how one goes from the standard curve to the concentration of the unknown. First, add a line of best fit, or Linear regression and display the equation on the chart. Ideally, the R2 value will be as close to 1 as possible. R represents the sum of the square values of the fit subtracted from each data point. Therefore, if R2 is much less than one, consider redoing the experiment to get one with more reliable data.[24] Graph 1. Actual BSA data attained from a micro scale UV-Vis Spectrophotometer The equation displayed on the chart gives a means for calculating the absorbance and therefore concentration of the unknown samples. In Graph 1, x is concentration and y is absorbance, so one must rearrange the equation to solve for x and enter the absorbance of the measured unknown.[25] It is likely that the unknown will have absorbance numbers outside the range of the standard. These should not be included calculations, as the equation given cannot apply to numbers outside of its limitations. In a large scale, one must compute the extinction coefficient using the Beer-Lambert Law A=εLC in which A is the measured absorbance, ε is the slope of the standard curve, L is the length of the cuvette, and C is the concentration being determined.[26] In a micro scale, a cuvette may not be used and therefore one only has to rearrange to solve for x. Table 1. Actual assay data for determine concentration of unknown based on line of best fit of the above standard curve In order to attain a concentration that makes sense with the data, the dilutions, concentrations, and units of the unknown must be normalized (Table 1). To do this, one must divide concentration by volume of protein in order to normalize concentration and multiply by amount diluted to correct for any dilution made in the protein before performing the assay. Alternative protein assays include: Ultraviolet–visible spectroscopy RGBradford Biuret protein assay Lowry protein assay BCA protein assay Amido black protein assay Colloidal gold protein assay ^ a b Ninfa, Alexander J; Ballou, David P; Benore, Marilee (2008). Fundamental Laboratory Approaches for Biochemistry and Biotechnology. Wiley. p. 113. ^ a b c d Bradford, Marion (1976). "A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" (PDF). Analytical Biochemistry. 72 (1–2): 248–254. doi:10.1006/abio.1976.9999. PMID 942051 – via Google Scholar. ^ "Quick Start TM Bradford Protein Assay" (PDF). www.bio-rad.com. ^ Bradford, Marion M. (May 1976). 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(2007), Environmental chemistry: fundamentals, p. 60, ISBN 978-0387260617 Bradford assay chemistry Variable Pathlength Spectroscopy OpenWetWare Retrieved from "   Bradford Plus Protein Assay Kit with Dilution-Free BSA Protein StandardsDetergent Compatible Bradford AssayBradford Protein Assay FeaturesReady-to-use Bradford assay kit with Dilution-Free BSA Protein Standards for reduced standard curve preparation timeIncreased linearity and reduced protein-to-protein variation compared to other commercial Bradford assay formulationsReady-to-use modification of the Bradford assay with additional additives to make it compatible with 1% or higher of commonly used detergents, including Triton X-100 and NP-40 detergentsReady-to-use formulation of the popular assay reagent originally described by Bradford in 1976Assay range (sample volume)125–1,500 µg/mL (5 µL) Or 1–25 µg/mL (150 µL)100–1,500 µg/mL (10 µL) or 2–25 µg/mL (150 µL)100–1,500 µg/mL (20 µL) or 1–25 µg/mL (150 µL)Compatible reagentsBuffer salts, metal ions, reducing agents, chelatorsDetergents, buffer salts, metal ions, reducing agents, chelatorsBuffer salts, metal ions, reducing agents, chelatorsIncompatibilitiesDetergents>5% detergent solutionsDetergentsIncubation time10 min10 min10 minProduct size950 mL/kit450 mL/kit950 mL/kitCat. No.A55866 Related Bradford Plus Kit:232362324623200Related product: Pierce Dilution-Free BSA Protein Standards, Multichannel Pipette Compatible, 2 mg/mLSelect Bradford assays now include Dilution-Free BSA Protein Standards which are a set of seven prediluted BSA standards, uniquely designed for multichannel pipette compatibility.The Pierce Dilution-Free protein standards minimize tedious dilutions and pipetting errors, delivering accurate results while reducing assay setup time. They are calibrated by direct comparison to purified BSA from the National Institute of Standards and Technology (NIST) to help ensure accurate standard curve generation. Bradford assay principles The Bradford assay was first described by Dr. Marion Bradford in 1976 and uses Coomassie G-250 dye in a colorimetric reagent for the detection and quantitation of total protein. Pierce Bradford Plus Protein Assays are modifications of the reagent first reported by Dr. Bradford.Chemistry of Coomassie-based Bradford protein assaysIn an acidic environment, proteins bind to Coomassie dye. This results in a spectral shift from the reddish-brown form of the dye (absorbance maximum at 465 nm) to the blue form (absorbance maximum at 610 nm). The difference between the two dye forms is greatest at 595 nm, making it the optimal wavelength to measure the blue color from the Coomassie dye–protein complex. If desired, the blue color can be measured at any wavelength between 575 nm and 615 nm. At the two extremes (575 nm and 615 nm), there is an approximate 10% decrease in the measured amount of color (absorbance) compared to that obtained at 595 nm. Development of color in Coomassie dye–based protein assays has been associated with the presence of certain basic amino acids—primarily arginine, lysine, and histidine—in the protein. Van der Waals forces and hydrophobic interactions also influence dye–protein binding. The number of Coomassie dye molecules bound to each protein is approximately proportional to the number of positive charges found on the protein. Free amino acids, peptides, and low molecular weight proteins do not produce color with Coomassie dye reagents. In general, the mass of a peptide or protein should be at least 3,000 Da for quantification with this reagent. In some applications, this can be an advantage.Figure 1. Reaction schematic for the Coomassie dye–based Bradford protein assays.The main disadvantage of Bradford protein assays is their incompatibility with surfactants at concentrations routinely used to solubilize membrane proteins. In general, the presence of a surfactant in the sample, even at low concentrations, causes precipitation of the reagent. This limitation can be overcome by using Detergent Compatible Bradford Assay. In addition, the Coomassie dye reagent is highly acidic, so proteins with poor acid-solubility cannot be assayed with this reagent. Finally, Coomassie reagents result in about twice as much protein-to-protein variation as copper chelation-based assay reagents. Product highlights The Bradford Plus Protein Assay Kit with Dilution-Free BSA Protein Standards reduces assay setup by eliminating tedious standard curve generation. Dilution-Free BSA Protein Standards are available as part of the Bradford Plus Protein Assay or available separately for use with any Pierce Bradford Assay.Figure 2. Bradford Plus Protein Assay with Dilution-Free BSA Protein Standards Assay Time.Pierce Detergent Compatible Bradford Assay Kit is a quick and ready-to-use modification of the well-known Bradford Assay for total protein quantitation. This formulation is compatible with up to 1% of commonly used detergents. Comparing Pierce Detergent Compatible Bradford Assay to the Bio-Rad DC Protein Assay, better sensitivity is seen with the Pierce Detergent Compatible Bradford Assay using common detergents. The range of the standard curve for the Pierce Detergent Compatible Bradford assay is 4 times broader than the range for the Bio-Rad DC assay.Figure 3. Bradford protein assay vs. the Bio-Rad DC protein assay. Each assay was performed in a microplate using BSA standards spiked with detergent or water (control), and followed the manufacturers' instructions.Table 1. Comparing Pierce Detergent Compatible Bradford Assay Kit with Bio-Rad DC Protein Assay Kit.FeaturesPierce Detergent Compatible Bradford Assay KitBio-Rad DC Protein Assay KitAssay measurement (absorbance maximum)595 nm750 nmTest tube assay sample volume50 µL100 µLMicroplate assay sample volume10 µL5 µLAssay working range100-1,500 µg/mL200-1,500 µg/mLAbsorbance range (sensitivity)HighLowNumber of reagents in kit1 reagent3 reagentsSetup time10 min30 minIncubation time10 min15 minTotal20 min45 min The Bradford assay quantifies protein concentration by measuring the color change of a dye, Coomassie Brilliant Blue G-250, from brown to blue. Bradford protein assays are colorimetric tests that determine the total amount of protein in solution against a known standard curve, usually bovine serum albumin (BSA). The assay principle relies upon the absorbance shift of the Bradford reagent, Coomassie Brilliant Blue G-250, when bound to proteins. Under acidic conditions, the free dye is in its cationic form, which appears reddish-brown, with a maximum absorbance at 470 nm. In the presence of proteins, it changes to its anionic form, as strong, noncovalent dye-protein complexes are created, resulting in a color change of the dye to blue, with a maximum absorbance at 595 nm. The degree of color change, as measured by optical density (OD), or absorbance, at 595 nm is directly proportional to the protein concentration, with higher absorbance values corresponding to greater total protein in solution. The Bradford method of protein quantification has some advantages over alternative methods, such as absorbance measurements at 280 nm. First, it utilizes readings in the visible spectrum range (595 nm). This allows for experiments to be conducted when UV spectrophotometers are unavailable. Additionally, the color change in the Bradford reagent is apparent by simple visual inspection. Compared to 280 nm quantification methods, Bradford assays are susceptible to different biases stemming from protein composition. That is, measurements of absorbance at 280 nm relies on the target protein containing side chains with aromatic rings, such as tryptophan, tyrosine and phenylalanine. On the other hand, the Van der Waals forces and hydrophobic interactions, which lead to the dye-protein complexes of the Bradford method, depend upon the presence of basic amino acids, such as arginine, lysine and histidine. Proteins which do not contain these side chains may invalidate each test method, respectively. One common limitation of the Bradford protein assay is its small dynamic range across which the method is linear. This is usually between 0 µg / mL to 10 µg / mL, beyond which measured dye OD no longer increases proportionally with protein concentration. The reason for this is due to the overlapping spectrums of the two forms of the Coomassie Brilliant Blue dye. One solution to this problem is to use the ratio of absorbances at 595 nm and 460 nm. This tends to yield a 10-fold increase in the dynamic range of the assay. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254. The Bradford assay is a fast and efficient colorimetric assay to determine the protein concentration in a wide range of solutions and is widely used for protein content of cell fractions and protein concentrations for gel electrophoresis. It is often done before an SDS-PAGE analysis. Using the reagent Coomassie Brilliant Blue G-250, the solution undergoes a visible change in color when the dye binds to proteins via van de Waals and electrostatic interactions, causing a shift from a dark red to a deep blue. This is a result of stabilization moving the absorbance maximum of the dye from 470 to 595 nm. Tested samples typically take less than five minutes to reach the indicative measures of 595 nm absorbance concentration, making the Bradford assay both reliable and quick. The Bradford assay is appropriate for general use and is distinguished from many other assays for being less susceptible to interference by compounds such as sodium, potassium, and certain carbohydrates that exist in samples. One note to remember is that sodium dodecyl sulfate (SDS), while often used to extract proteins from cells, interferes with the assay, and thus should be handled carefully. When proteins that carry residue of SDS are used as samples in the Bradford assay, the Coomassie dye is either kept from binding due to the bondage of SDS to proteins, or the SDS associates with the green form of the dye, shifting the equilibrium and overrepresenting the absorption at 595 nm regardless of true protein concentration.   The exact protein concentration can be determined due to interpolation from a standard curve made by measuring the absorbance of a dilution series of proteins with known concentrations within the response range of the Bradford assay. Bovine serum albumin and bovine γ-globulin are both common standards used for this assay. Materials in a Bradford assay include the color reagent and a protein standard. Kits can come with either pre-diluted protein standards of a listed concentration for a faster and easier use, or a lyophilized protein standard sample and heavily concentrated reagent for experimenters to manipulate and create their own concentration reagents and standards. A spectrophotometer with a maximum transmission at or around 595 nm is needed for the assay in order to determine the exact concentration in the tested sample solution. The Bradford assay does not work with proteins of all sizes - a minimum of 3000 Daltons in mass is a requirement for detection and measurement of concentration. The Bradford assay is generally recommended for the following sample types: Denaturing agents such as sodium thiocyanate, guanidine HCl, urea, and phenol Reducing agents such as dithiothreitol and β-mercaptoethanol Buffers, including HEPES, MES, MOPS, Tris, and phosphate Chelating agents such as aEDTA and EGTA Salts such as NaCl, KCl, and MgCl2 Tissue culture media such as Eadle's MEM and Hank's salt solution Sugars such as sucrose, glucose, and fructose DNA and RNA Glycerol Organic solvents such as acetone, methanol, and ethanol Detergents such as Triton™ X-100 and deoxycholate, and low concentrations of SDS Additional constraints of the Bradford assay include that it is incompatible with surfactants, proteins with poor acid solubility, and is highly sensitive to basic and aromatic amino acids such as arginine, histidine, and lysine. When completing this assay, you should also note to use cuvettes that are plastic and/or disposable, as glass and quartz cuvettes may be stained by the dye. Rely on G-Biosciences for Your Bradford Assay Needs At G-Biosciences, we offer a selection of supplies for your Bradford assay needs. Browse through our products and then contact us with any questions or to place an order. Protein quantification is a crucial step in many biochemical experiments, and the Bradford protein assay is one of the most widely used methods for this purpose.Principle of Bradford Protein AssayThe Bradford protein assay is based on the principle that the binding of a dye molecule, Coomassie Brilliant Blue, to proteins results in a shift in the dye's absorption spectrum. When the dye binds to protein, its absorption maximum shifts from 465 nm to 595 nm, resulting in a change in color from brown to blue. The amount of protein in a sample is determined by measuring the absorbance of the blue-colored solution at 595 nm and comparing it to a standard curve generated using known concentrations of protein.Why Perform Bradford Protein Assay?The Bradford protein assay is widely used because it is simple, rapid, and highly sensitive. It can detect as little as 1 microgram of protein in a sample, making it suitable for use with small amounts of protein, such as those obtained from cell culture or tissue samples. The assay is also compatible with a wide range of sample types, including serum, plasma, cell lysates, and purified protein solutions.Protocol of Bradford Protein AssayMaterials:Bradford reagentProtein sampleBovine serum albumin (BSA) standard solutionsSpectrophotometerCuvettesProcedure:Prepare the Bradford reagent according to the manufacturer's instructions. Briefly, the Bradford reagent can be prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol. Then, add 100 mL of 85% phosphoric acid and 850 mL of distilled water. Mix well and store the solution in a brown glass bottle.Prepare a series of BSA standard solutions of known concentrations (e.g. 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) by diluting a stock solution of BSA in distilled water.Dilute the protein sample to an appropriate concentration using distilled water. The concentration of the protein sample should be within the linear range of the standard curve.Add 20 µL of the protein sample or BSA standard to a cuvette containing 980 µL of distilled water. Then, add 1 mL of Bradford reagent to each cuvette and mix well by inverting the cuvette several times.Incubate the cuvettes at room temperature for 5 minutes. During this time, the Bradford reagent binds to the protein, resulting in a shift in the dye's absorption spectrum and a change in color from brown to blue.Measure the absorbance of the samples and standard at 595 nm using a spectrophotometer. Set the spectrophotometer to zero using a blank cuvette filled with distilled water. Then, measure the absorbance of the standard solutions and the protein sample.Calculate the protein concentration of the samples by comparing the absorbance values to the standard curve obtained from the BSA standard solutions. Plot the absorbance values of the standard solutions against their corresponding protein concentrations to create a standard curve. Use the standard curve to determine the protein concentration of the sample based on its absorbance.Repeat steps 4 to 7 for each sample and standard to obtain multiple measurements.Calculate the mean protein concentration of the samples by averaging the results of the multiple measurements.Tips for a Successful Bradford Protein Assay:Ensure that the Bradford reagent is freshly prepared and stored in a brown glass bottle to prevent photodegradation.Use distilled water to prepare all solutions and dilute the protein sample.Use BSA as a standard protein because it has a well-defined structure and is stable in solution.Measure the absorbance of the samples and standard within 5 to 10 minutes after adding the Bradford reagent, as prolonged incubation can lead to a decrease in the accuracy of the assay.Avoid high concentrations of detergents or reducing agents in the protein sample, as these can interfere with the binding of the dye to protein and affect the accuracy of the assay. * For Research Use Only. Not for use in diagnostic procedures. To estimate the concentration of protein using Bradford Method. The Bradford protein assay is a commonly used method for estimating the concentration of proteins in a sample. Generally, it is based on the binding of Coomassie Brilliant Blue (CBB) dye to proteins, resulting in a shift its maximum absorbance maximum from 465 nm to 595 nm. The orginal maxium absorbance of CBB is 465 nm and if it binds with the protein the absorbanc will be shifted to the 595nm resulting in a change in color from brown to blue. The Solution of CBB is originally brown and after linked with the protein molecule the color will be changed to blue color complex. The strong noncomplex bond is formed between carboxyl groups of the protein and dye by van der Waals force and amino group through electrostatic interactions. The amount of protein in a sample is determined spectrophotometrically by measuring the absorbance of the blue-colored solution at 595 nm and comparing it to a standard curve generated using known concentrations of protein. Fig: Estimation of protein by Bradford method Bradford reagent: Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol/Methanol, add 100 ml 85% (w/v) phosphoric acid. Make up to 1 liter after the dye has completely solubilized, and filter through filter paper before use. Protein Standard: 1 mg BSA/ml. Test tubes, Pipettes, Colorimeter, filter paper, funnel, etc., Prepare the aliquots of Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard BSA into the series of labeled test tubes. And, make up the volume to 1 ml in all the test tubes using distilled water. 1 ml of the given test sample in another test tube and labelled as "Test" and 1 ml of distilled water in another fresh tube and labeled as "blank". 1 ml of freshly prepared Bradford reagent is added to all the test tubes including the test tubes labeled 'blank' and 'test'. Mix the contents of the tubes uniformly by vertexing / shaking the tubes and incubate at room temperature for 10- 15 mins. Finally, the absorbance of the all-test tube is recorded at 540 nm against blank in the spectrophotometer. Observation Table: Fig: Estimation of protein by Bradford method (Observation Table) Calculate the protein concentration of the given test samples from the standard curve using the know concentration of BSA solutions. A standard curve is created by plotting the absorbance values of the standard solutions against their corresponding protein concentrations. Finally, the resultant standard curve is used to determine the protein concentration of the test sample based on its respective absorbance. he concentration of the given test solution can also be calculated using the given formula: The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. The Bradford is recommended for general use, especially for determining protein content of cell fractions and assesing protein concentrations for gel electrophoresis. Assay materials including color reagent, protein standard, and instruction booklet are available from Bio-Rad Corporation. The method described below is for a 100 µl sample volume using 5 ml color reagent. It is sensitive to about 5 to 200 micrograms protein, depending on the dye quality. In assays using 5 ml color reagent prepared in lab, the sensitive range is closer to 5 to 100 µg protein. Scale down the volume for the "microassay procedure," which uses 1 ml cuvettes. Protocols, including use of microtiter plates are described in the flyer that comes with the Bio-Rad kit. Principle The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range. In addition to standard liquid handling supplies a visible light spectrophotometer is needed, with maximum transmission in the region of 595 nm, on the border of the visible spectrum (no special lamp or filter usually needed). Glass or polystyrene (cheap) cuvettes may be used, however the color reagent stains both. Disposable cuvettes are recommended. Bradford reagent: Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol, add 100 ml 85% (w/v) phosphoric acid. Dilute to 1 liter when the dye has completely dissolved, and filter through Whatman #1 paper just before use. (Optional) 1 M NaOH (to be used if samples are not readily soluble in the color reagent). The Bradford reagent should be a light brown in color. Filtration may have to be repeated to rid the reagent of blue components. The Bio-Rad concentrate is expensive, but the lots of dye used have apparently been screened for maximum effectiveness. "Homemade" reagent works quite well but is usually not as sensitive as the Bio-Rad product. Warm up the spectrophotometer before use. Dilute unknowns if necessary to obtain between 5 and 100 µg protein in at least one assay tube containing 100 µl sample If desired, add an equal volume of 1 M NaOH to each sample and vortex (see Comments below). Add NaOH to standards as well if this option is used. Prepare standards containing a range of 5 to 100 micrograms protein (albumin or gamma globulin are recommended) in 100 µl volume. See how to set up an assay for suggestions as to setting up the standards. Add 5 ml dye reagent and incubate 5 min. Measure the absorbance at 595 nm. Prepare a standard curve of absorbance versus micrograms protein and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any. The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues. Obviously, the assay is less accurate for basic or acidic proteins. The Bradford assay is rather sensitive to bovine serum albumin, more so than "average" proteins, by about a factor of two. Immunoglogin G (IgG - gamma globulin) is the preferred protein standard. The addition of 1 M NaOH was suggested by Stoscheck (1990) to allow the solubilization of membrane proteins and reduce the protein-to-protein variation in color yield. Bradford, MM. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254. 1976. Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990). This assay is the only method that is non-destructive to protein samples (since the protein is not tied up in a colorimetric reaction). Is Bradford assay non destructive? The variation as a function of protein composition could be decreased by reaction at 60°C. Furthermore, the volume of reagents can be reduced, and it can be performed in 96-well plates. Like the Bradford method, this method is destructive to proteins. What is the Bradford assay? The Bradford assay uses standards to both quantify the amount of protein in samples and to subtract any background due to interfering substances that can shift the ratios between the three forms of the dye. The concentration range of standards in the kits cover the linear range of the Bradford assay. What are the limitations of the Bradford assay? The main limitation of the Bradford assay is its incompatibility with most detergents, routinely used to solubilize membrane proteins. (Interestingly, however, very low levels of non-ionic detergent, such as Triton X-100, may improve sensitivity and variability of the Bradford assay [25]). What is the principle of Bradford protein assay? The Bradford protein assay is used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue. How sensitive is the Lowry method? The Lowry method is sensitive to low concentrations of protein. Dunn [1992] suggests concentrations ranging from 0.10 – 2 mg of protein per ml while Price [1996] suggests concentrations of 0.005 – 0.10 mg of protein per ml. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. What is the best protein assay? Top 5 Protein Quantification Assays Bicinchoninic Acid (BCA) This colorimetric, two-step assay was originally developed in 1985 – making it a baby compared with the 64-year-old Lowry assay! Bradford. Folin-Lowry. Kjeldahl. Ultraviolet Absorption. How accurate is Bradford assay? The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. It is sensitive to about 5 to 200 micrograms protein, depending on the dye quality. How does SDS affect Bradford assay? The standard Bradford protein assay is insensitive to collagen. The addition of protein to a sub-threshold amount of SDS results in the formation of a green color measurable as an increase in absorbance at 700 nm, in contrast to the blue color measured at 595 nm in the standard assay. What is the color of the Bradford reagent before and after it reacts with protein? The Bradford reagent has Color is reddish brown with lambda max 470 nm. With proteins it develops blue color hence used for colorimetric estimation of proteins using Beer-Lambert law i.e. absorption is proportional to the concentration of the solution. Which protein is used in Lowry method? Lowry Method Lowry adds phosphomolybdic/phosphotungstic acid also known as Folin-Ciocalteu reagent. This reagent interacts with the cuprous ions and the side chains of tyrosine, tryptophan, and cysteine to produce a blue-green color that can be detected between 650 nm and 750 nm. What is the basic principle of Lowry method? Principle: The principle involved in Lowry method is determining the protein concentration by calculating the reactivity of the peptide nitrogen with the Copper ions under alkaline conditions followed by reduction reaction of Folinciocalteay phosphomolybdic phophotungstic acid to Heteropolymolybdenum blue by copper … Why are SDS added to Bradford dye binding protein assay? The addition of SDS to the Bradford dye-binding protein assay, a modification with increased sensitivity to collagen The standard Bradford protein assay is insensitive to collagen. How does the Bradford protein assay work how does it work? How the Bradford Protein Assay Works The Bradford protein assay is a time-tested colorimetric assay. When the Bradford reagent (acidified Coomassie Brilliant Blue G-250) binds to proteins, the dye undergoes a color change in the visible spectrum, with the absorbance maximum moving from 470 to 595 nm. What is the absorbance of the Bradford reagent? The Bradford protein assay is a time-tested colorimetric assay. When the Bradford reagent (acidified Coomassie Brilliant Blue G-250) binds to proteins, the dye undergoes a color change in the visible spectrum, with the absorbance maximum moving from 470 to 595 nm. What kind of protein assay is Bio-Rad? Bio-Rad Bradford protein assay kits include either of two different proteins, BSA or BGG, as standards to construct a standard curve for the relative quantitation of the proteins in the samples. For most determinations of protein concentration, relative values are generally sufficient.

Bradford Plus Protein Assay Kit with Dilution-Free BSA Protein StandardsDetergent Compatible Bradford AssayBradford Protein Assay FeaturesReady-to-use Bradford assay kit with Dilution-Free BSA Protein Standards for reduced standard curve preparation timeIncreased linearity and reduced protein-to-protein variation compared to other commercial Bradford assay formulationsReady-to-use modification of the Bradford assay with additional additives to make it compatible with 1% or higher of commonly used detergents, including Triton X-100 and NP-40 detergentsReady-to-use formulation of the popular assay reagent originally described by Bradford in 1976Assay range (sample volume)125–1,500 µg/mL (5 µL) Or 1–25 µg/mL (150 µL)100–1,500 µg/mL (10 µL) or 2–25 µg/mL (150 µL)100–1,500 µg/mL (20 µL) or 1–25 µg/mL (150 µL)Compatible reagentsBuffer salts, metal ions, reducing agents, chelatorsDetergents, buffer salts, metal ions, reducing agents, chelatorsBuffer salts, metal ions, reducing agents, chelatorsIncompatibilitiesDetergents>5% detergent solutionsDetergentsIncubation time10 min10 min10 minProduct size950 mL/kit450 mL/kit950 mL/kitCat. No.A55866 Related Bradford Plus Kit:232362324623200Related product: Pierce Dilution-Free BSA Protein Standards, Multichannel Pipette Compatible, 2 mg/mLSelect Bradford assays now include Dilution-Free BSA Protein Standards which are a set of seven prediluted BSA standards, uniquely designed for multichannel pipette compatibility.The Pierce Dilution-Free protein standards minimize tedious dilutions and pipetting errors, delivering accurate results while reducing assay setup time. They are calibrated by direct comparison to purified BSA from the National Institute of Standards and Technology (NIST) to help ensure accurate standard curve generation. Bradford assay principles The Bradford assay was first described by Dr. Marion Bradford in 1976 and uses Coomassie G-250 dye in a colorimetric reagent for the detection and quantitation of total protein. Pierce Bradford Plus Protein Assays are modifications of the reagent first reported by Dr. Bradford.Chemistry of Coomassie-based Bradford protein assaysIn an acidic environment, proteins bind to Coomassie dye. This results in a spectral shift from the reddish-brown form of the dye (absorbance maximum at 465 nm) to the blue form (absorbance maximum at 610 nm). The difference between the two dye forms is greatest at 595 nm, making it the optimal wavelength to measure the blue color from the Coomassie dye–protein complex. If desired, the blue color can be measured at any wavelength between 575 nm and 615 nm. At the two extremes (575 nm and 615 nm), there is an approximate 10% decrease in the measured amount of color (absorbance) compared to that obtained at 595 nm. Development of color in Coomassie dye–based protein assays has been associated with the presence of certain basic amino acids—primarily arginine, lysine, and histidine—in the protein. Van der Waals forces and hydrophobic interactions also influence dye–protein binding. The number of Coomassie dye molecules bound to each protein is approximately proportional to the number of positive charges found on the protein. Free amino acids, peptides, and low molecular weight proteins do not produce color with Coomassie dye reagents. In general, the mass of a peptide or protein should be at least 3,000 Da for quantification with this reagent. In some applications, this can be an advantage.Figure 1. Reaction schematic for the Coomassie dye–based Bradford protein assays.The main disadvantage of Bradford protein assays is their incompatibility with surfactants at concentrations routinely used to solubilize membrane proteins. In general, the presence of a surfactant in the sample, even at low concentrations, causes precipitation of the reagent. This limitation can be overcome by using Detergent Compatible Bradford Assay. In addition, the Coomassie dye reagent is highly acidic, so proteins with poor acid-solubility cannot be assayed with this reagent. Finally, Coomassie reagents result in about twice as much protein-to-protein variation as copper chelation-based assay reagents. Product highlights The Bradford Plus Protein Assay Kit with Dilution-Free BSA Protein Standards reduces assay setup by eliminating tedious standard curve generation. Dilution-Free BSA Protein Standards are available as part of the Bradford Plus Protein Assay or available separately for use with any Pierce Bradford Assay.Figure 2. Bradford Plus Protein Assay with Dilution-Free BSA Protein Standards Assay Time.Pierce Detergent Compatible Bradford Assay Kit is a quick and ready-to-use modification of the well-known Bradford Assay for total protein quantitation. This formulation is compatible with up to 1% of commonly used detergents. Comparing Pierce Detergent Compatible Bradford Assay to the Bio-Rad DC Protein Assay, better sensitivity is seen with the Pierce Detergent Compatible Bradford Assay using common detergents. The range of the standard curve for the Pierce Detergent Compatible Bradford assay is 4 times broader than the range for the Bio-Rad DC assay.Figure 3. Bradford protein assay vs. the Bio-Rad DC protein assay. Each assay was performed in a microplate using BSA standards spiked with detergent or water (control), and followed the manufacturers' instructions.Table 1. Comparing Pierce Detergent Compatible Bradford Assay Kit with Bio-Rad DC Protein Assay Kit.FeaturesPierce Detergent Compatible Bradford Assay KitBio-Rad DC Protein Assay KitAssay measurement (absorbance maximum)595 nm750 nmTest tube assay sample volume50 µL100 µLMicroplate assay sample volume10 µL5 µLAssay working range100-1,500 µg/mL200-1,500 µg/mLAbsorbance range (sensitivity)HighLowNumber of reagents in kit1 reagent3 reagentsSetup time10 min30 minIncubation time10 min15 minTotal20 min45 min