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Determination of Protein Using Microdrop Assay

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Determination of Protein Using Microdrop Assay

A Project

Submitted

To

Governors State University

By

Hardik Khared

In the partial fulfillment of the

Requirements of degree

Of

Master of Science

In

Analytical Chemistry

May 2011

Governors State University

University Park, IL

DEDICATION

To my mother

Shardaben B Khared

And to my father

Babubhai K. Khared

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This project would never been possible without the guidance and the help of *Dr. Walter A. Henne*. His experience, knowledge, persistent, and motivation help me in all my research and paper work. Without him and his lab this project has never been possible. So I would special thanks to my Advisor *Dr. Walter A. Henne*.

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Abstract

After purification of protein, it is important to know the concentration of protein in our samples. Concentration or amount of protein in the sample is determined by different assay as BCA (bicinchoninic acid) Assay, Bradford Assay, and Lowery Assay. There are different methods available to perform these assays, which have some limitations, restrictions, advantages and disadvantages. These methods require large amounts of costly reagents, proteins and most importantly, valuable time. To overcome these problems a new method is developed called Microdrop Protein Analysis. This new method required a very small amount of reagents and protein mixtures (2-5 μl volume) and is very quick and easy to perform. The main objective of this project was to develop either new or modified methods for detection and analysis of proteins using a newly introduced micro drop plate reader equipped with a 16 well microdrop reader (Take3 Plate). This Take3 Multi-Volume plate allows for easy and rapid analysis of 16 samples having as low as 2- μl volumes each by using Gen 5 software¹. The plate used in the instrument EpochTM micro plate spectrophotometer. We used commercially available BSA (bovine serum albumin) Protein as our test moiety. The concentration of protein in the solution was determined by BCA (bicinchoninic acids) Assay using UV-280 protocol or microdrop micro-BCA protocol provided by the manufacture (Pierce Chemical Company) with the appropriate controls (PBS blank and BSA test control protein). First, the experiment was carried out at room temperature to determine the lowest concentration of protein that we can use and get linear curve. Then, we did time-dependent studies at room temperature and heated the plate in a humidified incubator before measuring the absorbance. Then, we plotted a graph of concentration on the X-axis and absorbance on Y-axis to find out the concentration of protein. The overarching strategy is to develop robust assays that use minimum sample amounts given the high cost and limited

availability of many proteins. Successful completion of this work will benefit protein researches in rapid identification and analysis of protein during expression, analysis and isolation.

Introduction

Accurate determination of protein concentration in samples has since long been critically importance in biochemical laboratories for the study of many biochemical processes. To determine accurate concentration of protein, different assays are available as a) Colorimetric Assay: Biuret, Bradford, BCA, Modified Lowery and b) Absorbance Assay: Absorbance at 280 nm, Absorbance at 205 nm, Extinction Coefficient². There are currently wide ranges of different methods available for estimating protein concentration in a sample. The different methods used to perform this assay consume more proteins sample and expansive reagents. So, we performed this experiment on microdrop level to save proteins and reagents. This microdrop method used only 2-5 μ l of protein-reagent mixture and it was very quick to perform. In this experiment, we use Take3 plate to perform the BCA and Bradford Assay. This colorimetric assay gives more accuracy and is easier to perform on microdrop levels. They are as follows:

Bradford Assay:

In 1976, Dr. Marion Bradford et.al invented the Bradford Assay³. It's a very popular Dye-binding protein assay for detection and quantitation of protein in samples. Coomassie Plus Brilliant Blue G-250 dye is used as the coloring reagent in this assay. In 1985, Campton and Jones found that Coomassie Plus Brilliant Dye G-250 was available in three different forms: Cationic form (red color 470nm), neutral form (green color, 650 nm), and anionic form (blue color, 595 nm)⁴. The Bradford reagent reacts with amino acid residues at arginine, tyrosine, tryptophan, phenylalanine and histidine in the amino acid sequence of the protein³. The Protein is

directly bound to the Coomassie Plus G-250 dye in the acidic environment of the dye. As protein concentration increases it binds more-and-more with dye and the color of the solution becomes darker. Absorbance of this solution is measured at 592nm in a microplate reader to find the concentration of protein in the solution⁵.

Chemistry of Bradford Assay:

When Coomassie Plus Dye binds with protein, its absorbance maxima of the acidic environment shifts from a lower wavelength (465nm) to a higher wavelength (595nm)⁶. Color of the dye has been changed from its reddish-brown form to blue form⁷. This blue color complex is measured at a wavelength of 592nm in the microplate reader. Intensity of this color formation is based up on the presence of basic amino acid in the protein. This complex formation involves two types of bond interactions. In first step bond reactions dye donates its free electron to the protein, which exposes the protein hydrophobic pockets. In second steps, through Van Der Waals Forces, these hydrophobic pockets bind with non-polar regions of the dye. This binding helps in stabilizing the blue color complex, which measured at 592nm⁶.

Advantages of Bradford Assay:

- It's easy and quick to perform
- Only one reagent (stable for 12 months) is required for performing the entire assay
- No special instrument is required because it carries out at a room temperature
- The micro assay requires minimal sample amounts. Sample to reagent ratio is 1:1 in a micro assay that is quite smaller than the 96-plate assay (1:50 reagent-ratio) and working linear range of this assay is 125 -1000 $\mu\text{g}/\text{ml}$ ⁸.
- Reagent is ready to use; no further dilution and filtration is required.
- Assay is compatible with a wide range of metals ions, buffer salts and chelating agents⁸.

Disadvantages of Bradford Assay:

- The main disadvantage is that it is not compatible with surfactants means at low concentration of it precipitating the reagent.
- The Micro Bradford Assay response is not linear as BCA Assay; minimum mass of protein required is 3000 Daltons⁸.
- Presence of detergent interfered with the result of assay.

Bicinchoninic (BCA) Based Assay:

The BCA Assay is a colorimetric assay invented by Paul K Smith, et.al, in 1985⁹. Since then, it has been a popular method for determination of protein concentration because it is very easy to use, very sensitive and forms more stable complexes than any other colorimetric assay. The BCA reagent more likely reacts with amino acid residues of tyrosine, cysteine and tryptophan in the amino acid sequence of protein, resulting in development of color complex¹⁰. The intensity of color formation has been increased with increased protein concentration in a sample¹¹.

Chemistry of BCA Assay:

Two reactions are taking place in BCA Assay. First, is a reduction of Cu^{2+} to Cu^{1+} by protein in alkaline medium (Biuret reaction) from a light blue color complex⁷. It shows that the reduction of copper ion is proportional to the amount of protein present in the sample. In the second step, reduce cuprous cation (Cu^{1+}) reacts with two moles of bicinchoninic acid and forms purple color solution that absorbed at 562nm¹². Both reactions are shown at the end of the thesis. As a result the water-soluble complex is formed. The complex shows the strong absorbance at 562 nm, which has been linear with increased protein concentration in the sample. Thus, the BCA Assay has determined the quantity of protein⁷.

Advantage of BCA Assay:

- BCA reagent is stable at room temperature for 24 hours⁸.
- Easy to perform and provides, large linear operating range, assay is well suited with many detergents.
- It gives linear response means, as protein concentration increases, intensity of color formation was increased.
- The BCA Assay provides high sensitivity and less protein-protein variation. This assay is performed with peptide and proteins.

Disadvantage of BCA Assay:

- The BCA Assay is not compatible with reducing agents; presences of these agents interfere in the accurate determination of protein⁸.
- Sometime it is necessary to dilute the concentrated protein before performing the assay.

Materials and Reagents

The list of materials and reagents are needed for this project include: Bovine Serum Albumin (Protein Sample) by Sigma Aldrich Lot No. 029K1394, Phosphate-Buffered Saline (PBS) by Mediatech Inc. Lot No. 210404174, Bicinchoninic Acid Kit by Sigma Aldrich in that Bicinchoninic Acid solution Batch No. 118K5300, Copper (II) Sulfate solution Batch No. C2284, Parafilm "M", Bradford Reagent Coomassie Plus Protein Assay Reagent by Thermo Scientific, Lot # KD 132217.

Instrumentation

This project used an Epoch Multi Volume Spectrophotometer System by BioTek, having EpochTM Microplate Spectrophotometer and Take3TM Multi Volume Plate, Humidified Incubator, and Raining Micropipettes.

Methodology

Bradford Assay:

First we made stock solution of the protein sample by dissolving 20 mg of BAS protein into 10 ml of PBS solution. From the stock solution, we made serial dilutions of 2 mg/ml, 1.5 mg/ml, 1.0 mg/ml, 0.75 mg/ml, 0.5 mg/ml, 0.25 mg/ml and 0.125 mg/ml (shown in figure 11). We also had one blank sample i.e. only PBS, no protein solution added. The Bradford reagent Coomassie Dye G-250 was available, as a ready to use formula and did not require any further dilutions. We can mix the Bradford reagent and protein sample directly onto microplate spots, but here we mixed them on parafilm "M" (as shown in figure 13) before putting them on the plate to get better results. Now we transferred 5 μ l of that mixture to Take3 plate as shown in figure 14. Then, we put that microplate plate in the EpochTM microplate spectrometer and used 'Gen 5' software to determine the absorbance of protein samples at 592nm. Finally, we plotted a graph of absorbance versus concentration of solutions.

This experiment was performed at room temperature and also as a time dependent study based on room temperature and humidified incubator.

Determination of protein at a room temperature:

First step was to place 6 μl of blank and diluted protein sample onto parafilm

Then, place 6 μl of Coomassie Dye G-250 on each of the eight drops.

Wait for 5 minute until development of blue color in mixtures.

Then pipett out the 5 μl of mixture to Take3 microplate and made duplicates of it.

Place the plate in the spectrophotometer.

Gen-5 software was used to measure the absorbance at 592 nm.

Time dependent study at room temperature:

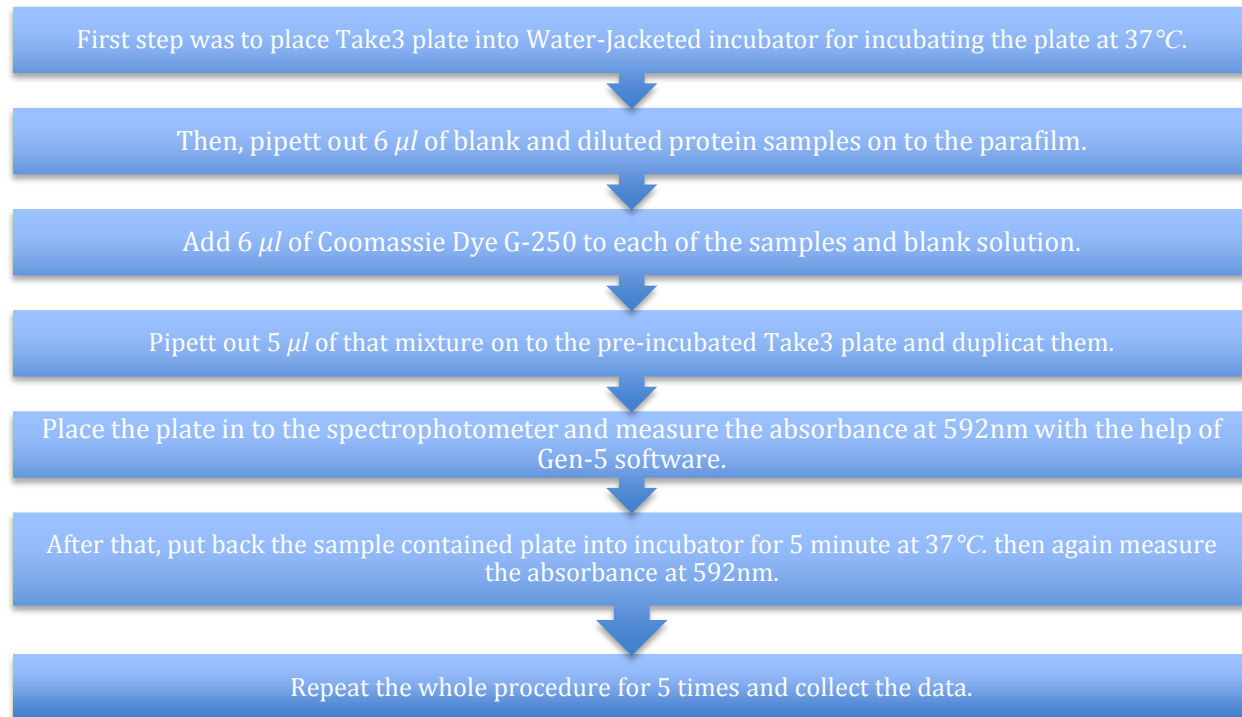
First, pipett out 6 μl of blank and diluted protein samples onto parafilm.

Add 6 μl of Coomassie Dye G-250 to each of the eight samples.

Then, pipett out 5 μl of each mixtures to the take3 plates and duplicate each of them.

Place the plate in the spectrophotometer and measure the absorbance at 592nm.

After that, measure the absorbance at 5-minute interval for 30 minutes at room temperature and collect the absorbance data.

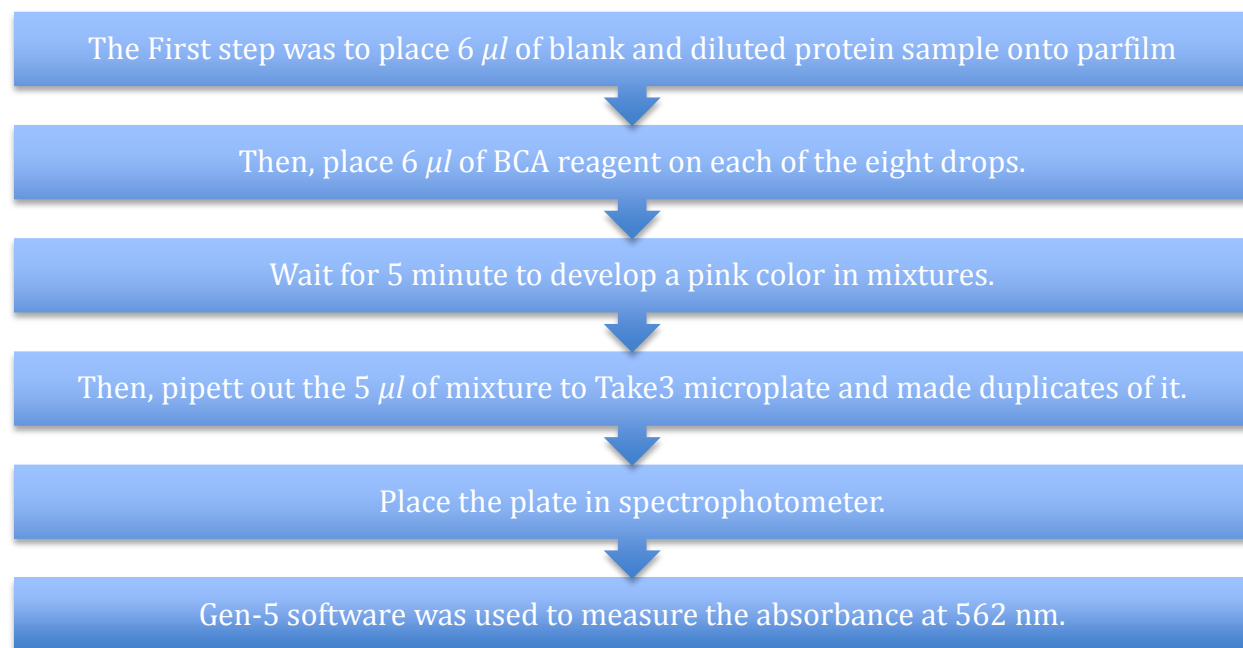
Time dependent study by incubation in Humidified Incubator:**BCA Protein Assay:**

The Bicinchoninic acid kit has two solutions, copper (II) sulfate solution and Bicinchoninic acid solution. The proportion of these two solutions is very important to perform the BCA Assay. Appropriate proportion bicinchoninic acid solutions and copper (II) sulfate solutions are a 50:1 ratio, respectively. In this experiment, we used 490 μ l of bicinchoninic acid and 10 μ l of copper (II) sulfate solution to make up the volume of 500 μ l.

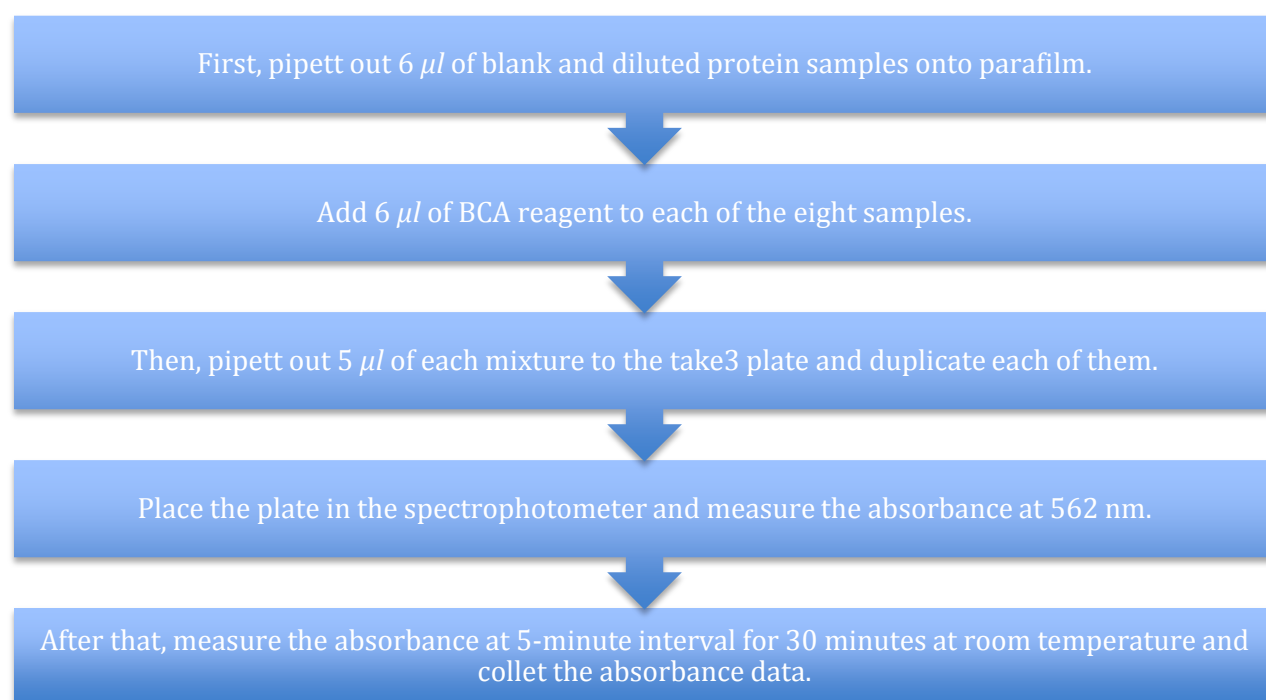
Bovine serum albumin was used as a protein test sample. We first dissolved 20 mg of BSA protein in 10 ml of PBS solution. From that stock solution, we made a serial dilution of 2 mg/ml, 1.5 mg/ml, 1.0 mg/ml, 0.75 mg/ml, 0.5 mg/ml, 0.25 mg/ml and 0.125 mg/ml as shown in figure 12. We also had one blank sample (i.e. only PBS no protein solution was added). We did this experiment in three different ways 1) at room temperature 2) as a time dependent study at

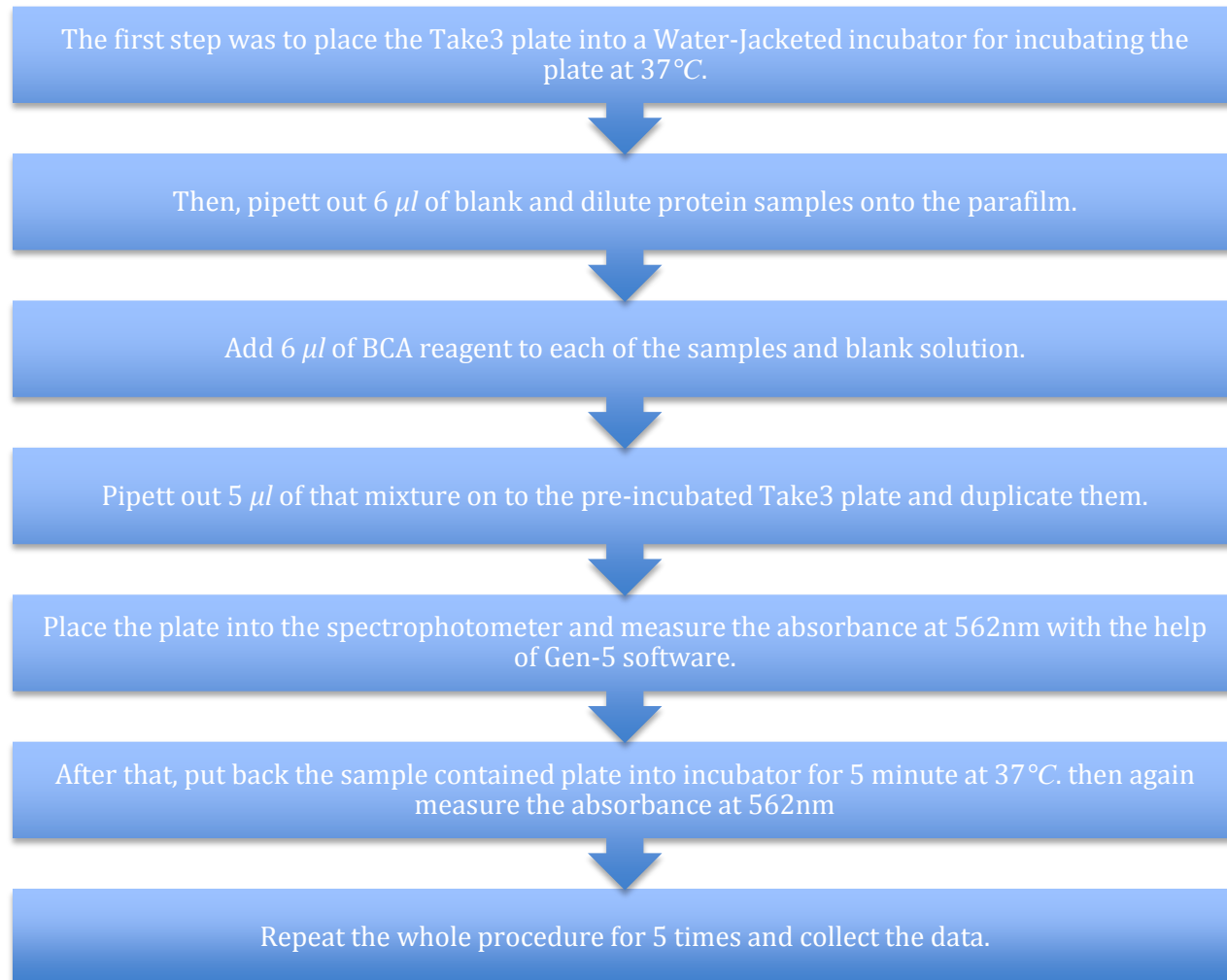
room temperature and 3) as a time dependent study in a humidified incubator and measured the absorbance at 562 nm.

BCA Assay at a room temperature:



Time dependent study at room temperature:



Time dependent study by incubation in Humidified Incubator:**Results**

Below, are the tables for Bradford and BCA Assay performed at room temperature. In this method, I did several trials to find out the minimum concentration of protein sample that can be determined by Bradford and BCA Assay. Below, the tables display the data of low protein concentration versus absorbance (after subtracted from blank) and also different times used for development of color.

Table 1, displays results of the Bradford Assay at very diluted protein sample.

Table-1 Bradford Assay at low concentration.

Protein Concentration (mg/ml)	Absorbance after 15 minutes (After subtracting from the blank) at 592 nm
0	0
0.0625	0.002
0.125	0.029
0.25	0.03
0.5	0.039
0.75	0.069
1	0.078
1.5	0.09

Table 2 displays results of the Bradford Assay after keeping the mixture on parafilm “M” for 15 minutes at room temperature.

Table-2 Bradford Assay after 15 minute at room temperature (Omitted 0.0625 mg/mL)

Protein Concentration (mg/ml)	Absorbance after 15 minutes (After subtracting from Blank) at 592 nm
0	0
0.125	0.023
0.25	0.032
0.5	0.049
0.75	0.053
1	0.059
1.5	0.061
2	0.068

Table 3 display results of the Bradford Assay after settling down the mixture on parafilm “M” for 30 minutes at room temperature.

Table-3 Bradford Assay after 30 minute at room temperature.

Protein Concentration (mg/ml)	Absorbance after 30 minutes (After subtracting from blank) at 592 nm
0	0
0.125	0.027
0.25	0.039
0.5	0.05
0.75	0.057
1	0.061
1.5	0.067
2	0.08

Table 4 displays results of the BCA Assay for the diluted protein sample.

Table-4 BCA Assay at Low concentration.

Protein Concentration (mg/ml)	Absorbance 20 minute (After subtracting from the blank) at 562 nm
0	0
0.0625	0.001
0.125	0.026
0.25	0.032
0.5	0.043
1	0.082
1.5	0.115

Table 5 and table 6 are displays results of the BCA Assay after keeping the mixture on parafilm “M” for 10 minutes and 30 minutes respectively, at room temperature.

Table-5 BCA Assay after 10 minute at room temperature. (Omitted 0.0625 mg/mL)

Protein Concentration (mg/ml)	Absorbance after 10 minute (After subtracting from the blank) At 562 nm
0	0
0.125	0.017
0.25	0.027
0.5	0.051
0.75	0.07
1	0.093
1.5	0.129
2	0.139

Table-6 BCA Assay after 25 minute at room temperature.

Protein Concentration (mg/ml)	Absorbance after 25 minute (After subtracting from the blank) At 562 nm
0	0
0.125	0.019
0.25	0.03
0.5	0.05
0.75	0.07
1	0.1
1.5	0.14
2	0.16

Table 7 and table 8 displays results of the Bradford Assay and BCA Assay performed at room temperature by 5-minute time interval for measurements of absorbance, respectively.

Table-7 Bradford Assay conducts at room temperature at 5 minutes interval.

Concentration (Mg/ml)	Absorbance (After subtracting from blank) Measured at 592 nm						
	0 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
0	0	0	0	0	0	0	0
0.125	0.019	0.03	0.033	0.037	0.038	0.04	0.044
0.25	0.023	0.035	0.038	0.04	0.045	0.049	0.056
0.5	0.045	0.053	0.059	0.065	0.07	0.074	0.08
0.75	0.059	0.07	0.073	0.078	0.081	0.087	0.092
1	0.069	0.073	0.077	0.081	0.086	0.091	0.099
1.5	0.07	0.088	0.08	0.086	0.091	0.098	0.104
2	0.082	0.093	0.096	0.101	0.107	0.113	0.119

Table-8 BCA Assay conducts at room temperature at 5-minute interval.

Concentration (Mg/ml)	Absorbance (After subtracting from blank)						
	Measured at 562 nm						
	0 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
0	0	0	0	0	0	0	0
0.125	0.022	0.028	0.03	0.033	0.034	0.034	0.035
0.25	0.022	0.036	0.04	0.045	0.048	0.049	0.051
0.5	0.029	0.047	0.052	0.06	0.064	0.066	0.068
0.75	0.038	0.054	0.064	0.08	0.086	0.089	0.089
1	0.043	0.065	0.079	0.093	0.1	0.104	0.109
1.5	0.047	0.098	0.117	0.136	0.146	0.153	0.161
2	0.057	0.114	0.138	0.164	0.178	0.188	0.2

Table 9 and table 10 display results of Bradford Assay and BCA Assay performed by incubating microplate in a humidified incubator for 5 minutes time interval for measurement of absorbance, respectively.

Table-9 Bradford Assay 5 minutes interval study by incubating the plate.

Concentration (Mg/ml)	Absorbance (After subtracting from blank)						
	Measured at 592 nm						
	0 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
0	0	0	0	0	0	0	0
0.125	0.011	0.025	0.029	0.03	0.032	0.033	0.035
0.25	0.019	0.04	0.046	0.049	0.053	0.055	0.059
0.5	0.04	0.046	0.05	0.056	0.059	0.064	0.068
0.75	0.049	0.056	0.062	0.064	0.07	0.079	0.083
1	0.061	0.067	0.076	0.079	0.083	0.089	0.095
1.5	0.071	0.079	0.082	0.087	0.091	0.099	0.106
2	0.078	0.089	0.095	0.104	0.112	0.119	0.121

Table 10 BCA Assay 5 minutes interval study by putting microplate in incubator.

Concentration (Mg/ml)	Absorbance (After subtracting from blank)						
	Measured at 562 nm						
	0 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
0	0	0	0	0	0	0	0
0.125	0.011	0.017	0.019	0.022	0.023	0.024	0.025
0.25	0.027	0.023	0.029	0.035	0.038	0.04	0.041
0.5	0.018	0.039	0.045	0.047	0.051	0.055	0.064
0.75	0.038	0.057	0.066	0.076	0.084	0.086	0.087
1	0.073	0.075	0.086	0.099	0.106	0.111	0.119
1.5	0.083	0.1	0.12	0.141	0.149	0.156	0.163
2	0.119	0.139	0.16	0.189	0.19	0.204	0.219

Discussions

Bradford Assay:

Coomassie plus dye G-250 is available in three different forms, so that the absorption spectrum of dye was overlapping and caused non-linear responses of samples⁴. Figure 1 shows that at very dilute concentrations (0.0625 mg/ml), the response was very low and non-linear. Figure 2 and Figure 3 show that the response was not linear at lower concentration (0.125 and 0.250 mg/ml) but as the concentration (1.5 and 2.0 mg/ml) increased it become linear. Figure 7 shows that as the time increased more of the BSA proteins reacted with Bradford reagent. In dilute solution of 2 mg /ml at 0 minute absorbance intensity was 0.089 which increased to 0.129

at 30 minutes. So, from the time-dependent studies shows that time did not give much effect to absorbance intensity. It occurs to all of the dilutions. Figure 9 shows that the response was non-linear with increasing time by incubating the microplate in the incubator. Result from the both time-dependent studies shows that there was a very negligible difference in absorbance intensity between the two methods

BCA ASSAY:

The BCA Assay was a quick, easy, very sensitive and popular assay for determination of protein. Figure 4 shows that at very dilute protein solution (0.0625-mg/ml), absorbance intensity was very low 0.001. Thus, it was found that a very dilute protein solution was not detected by the microdrop BCA Assay. Figure 5 shows that after 10 minute at low concentration, the line was non-linear but it becomes linear after 0.5-mg/ml dilutions. However in figure 6 show that after 25 minute at very diluted solutions (0.125, 0.25 mg/ml) the response of absorbance was also linear. Figure 8 shown that the times effect the intensity of color formation. Increased time increased the absorbance intensity of the serial dilution of protein sample. At 0 minute the absorbance of 2-mg/ml solutions was found to be 0.057, which increased to 0.2 at a time of 30 minutes. This value was doubled than what we saw in the Bradford Assay, it proves that at low concentration formation of complex was more stable in BCA than the Bradford Assay. Figure 10 shows that increased time for incubating the microplate in the incubator also increases absorbance intensity for the serial diluted sample. However, there was no significant difference between the experiment conducted at room temperature and at incubating the microplate in the incubator.

Conclusions

We successfully developed linear curve in the BCA Assay at microdrop level. However the Bradford Assay did not give a linear curve at this lower microdrop level. In the Bradford Assay it was determined that at low standard concentrations the response was non-linear but as the concentration was increased the response became linear. Also, in BCA Assay the intensity of absorbance was increased as time increased but Bradford Assay was ineffective in a time-dependent study. Results from figure 7, 8 and figure 9, 10 shows that it wasn't necessary to incubate the microplate in incubator. Atmospheric moisture does not have much effect on the absorbance intensity of protein sample. Figure 1 and 4 shows that at very dilute solution (0.0625 mg/ml), the assay did not give linear response. Thus minimum detection limit for micro BCA Assay was found to be 0.1 mg/ml. By performing these micro assays we avoid using large amounts of protein samples and reagents used for the detection of protein concentration.

Future Studies

Future Studies will be the lower the samples volume from 5 μ l to 2 μ l. This helps in further reduce of the requirements of proteins and reagents. Also future study will be on the increase sensitivities of Microdrop assay for very lower concentrations. Test will perform with more users to establish user-to-user variability.

Acknowledgement

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Reference

1. <http://www.biotek.com/products/accessories/?accid=10186>
2. <http://www.ruf.rice.edu/~bioslabs/methods/protein/protein.html>
3. <http://www.piercenet.com/products/browse.cfm?fldID=02020105>
4. http://kirschner.med.harvard.edu/files/protocols/BioRad_proteinassay.pdf
5. Ernst O, Zor T. Linearization of the bradford protein assay. *J Vis Exp*. 2010(38).
6. Thermo Scientific Pierce Protein Assay Technical Handbook version 2 by Thermo Scientific
7. Zor T, Selinger Z. Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. *Anal Biochem*. May 1 1996;236(2):302-308.
8. http://www.cultek.com/inf/otros/perfil-provedores/Pierce_manual_protein_assay.pdf
9. http://www.criver.com/SiteCollectionDocuments/qc_mm_r_BCA_package_insert.pdf
10. <http://www.piercenet.com/browse.cfm?fldID=02020101>
11. Walker JM. The bicinchoninic acid (BCA) assay for protein quantitation. *Methods Mol Biol*. 1994;32:5-8.
12. Bainor A, Chang L, McQuade TJ, Webb B, Gestwicki JE. Bicinchoninic acid (BCA) assay in low volume. *Anal Biochem*. Mar 15 2011;410(2):310-312.

Figure 1 Bradford Assay at low standard.

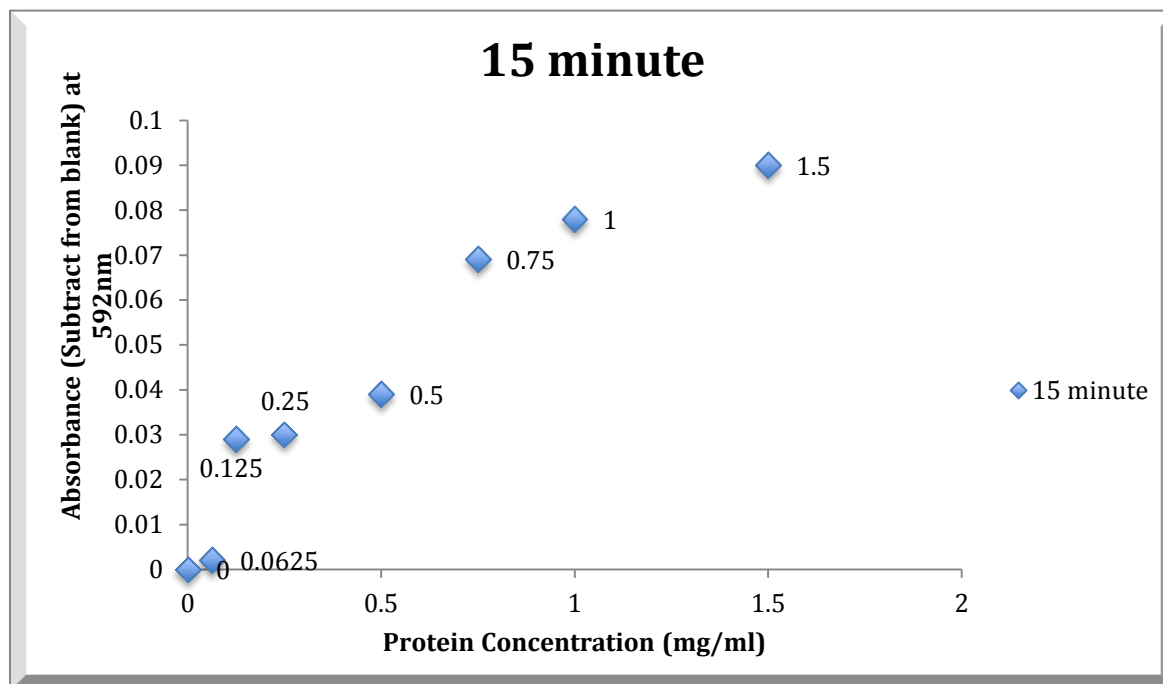


Figure 2 Bradford Assay after 15 minute at room temperature.

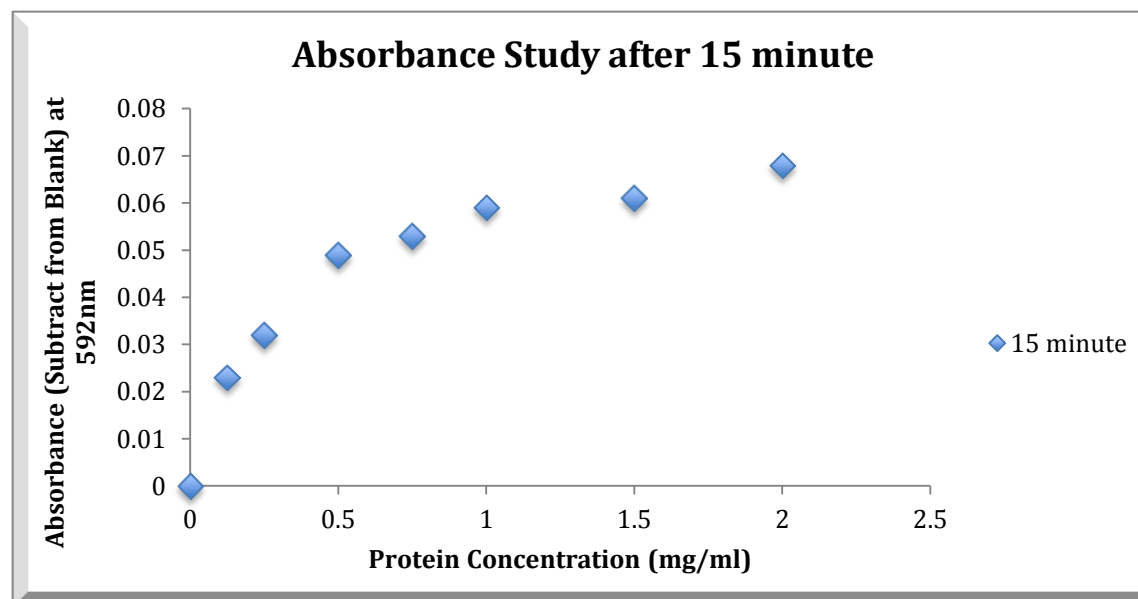


Figure 3 Bradford Assay after 30 minute at room temperature.

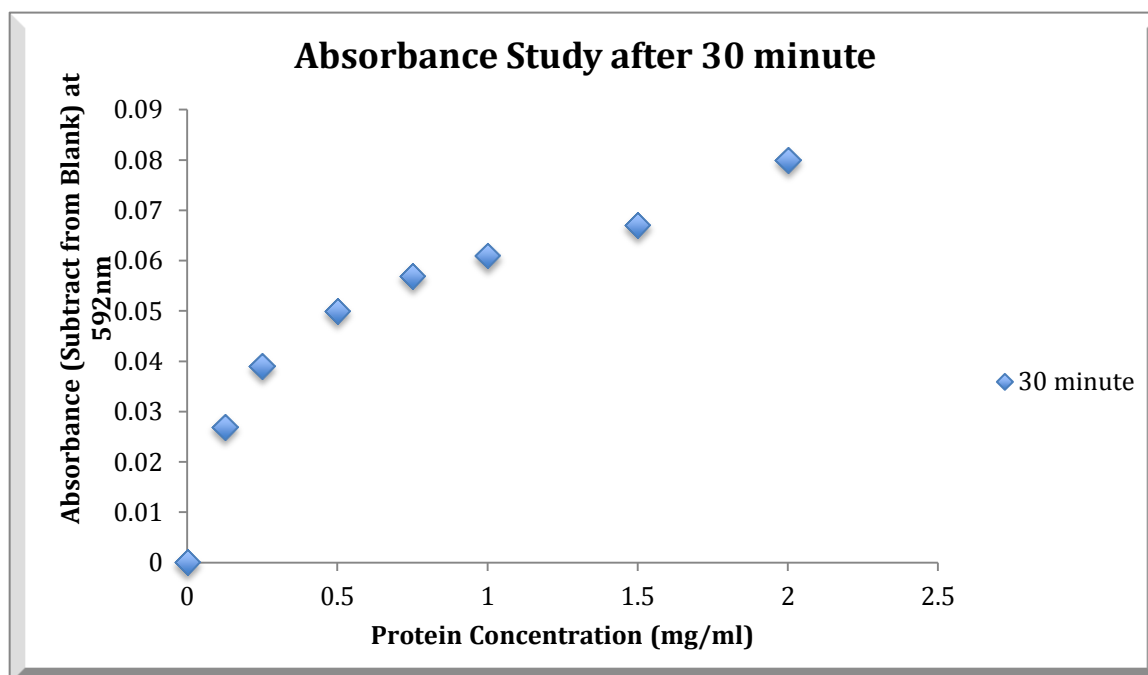


Figure 4 BCA Assay low standards.

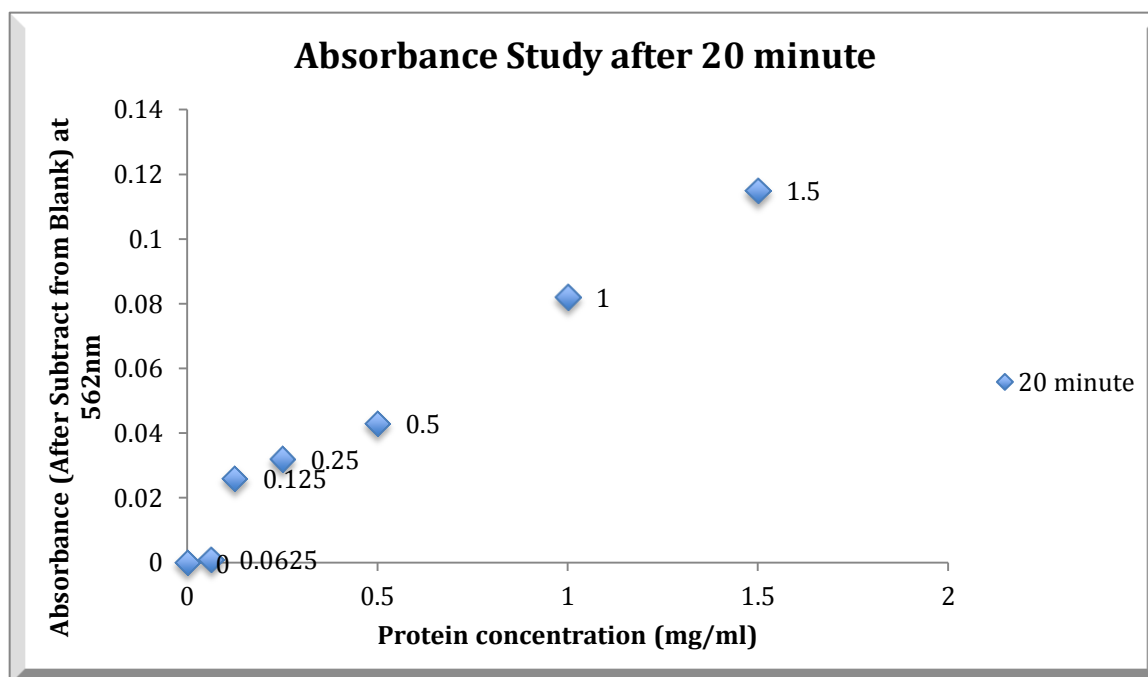


Figure 5 BCA Assay after 10 minute at room temperature.

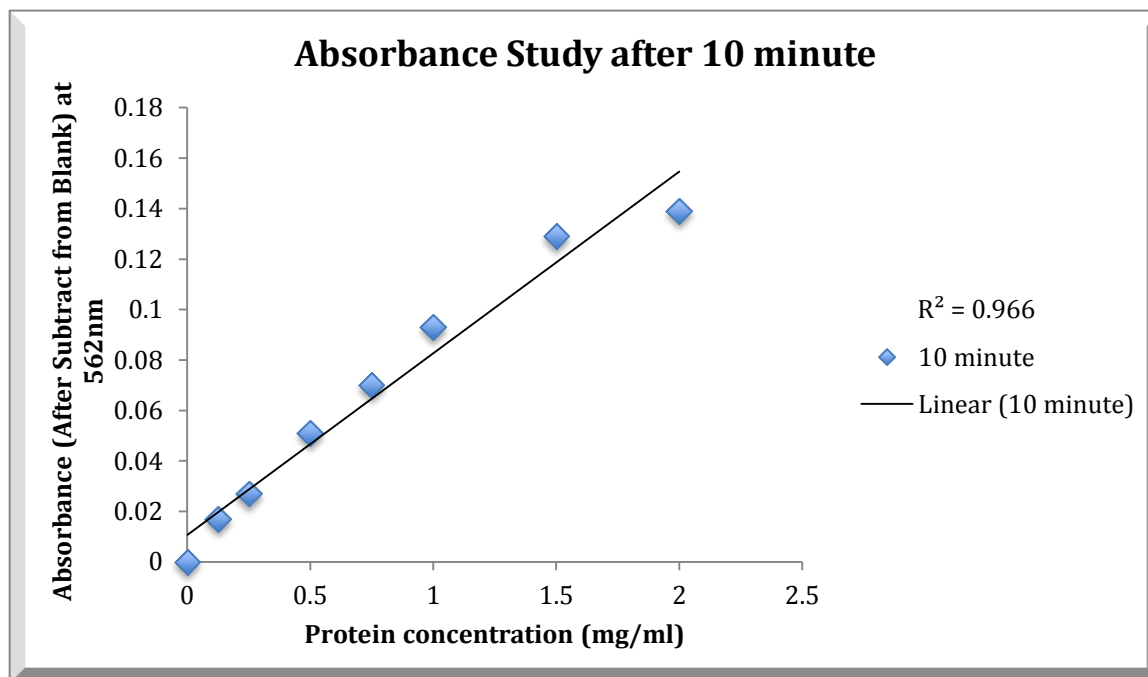


Figure 6 BCA Assay after 25 minute at room temperature.

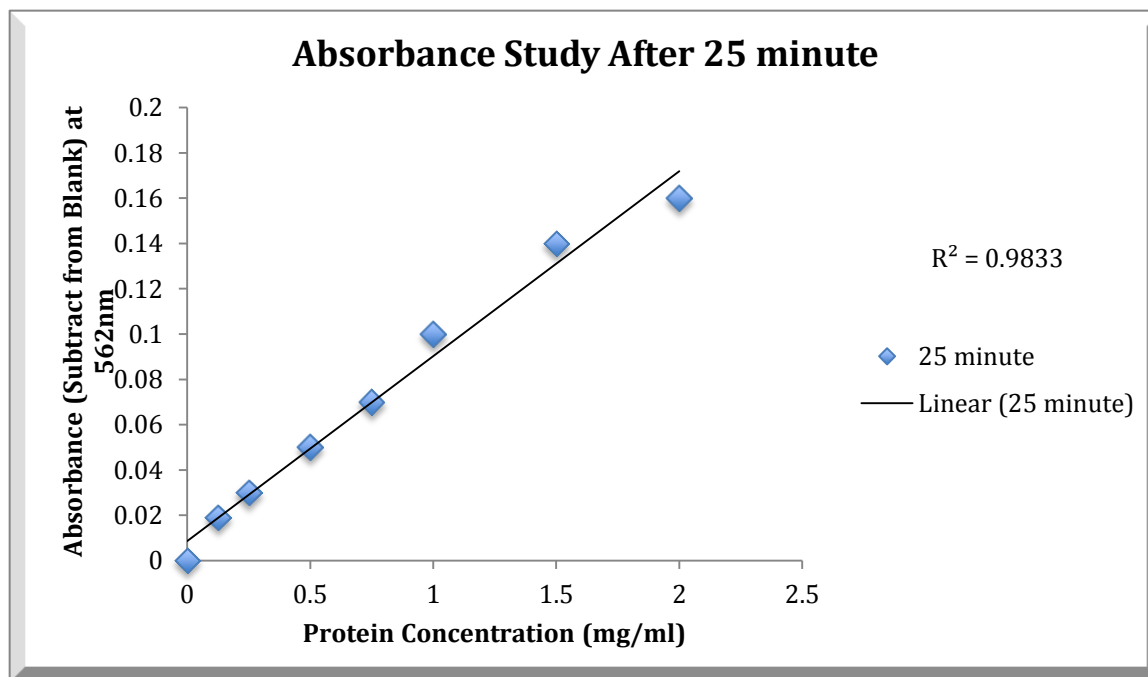


Figure 7 Bradford Assay conduct at room temperature at 5 minutes interval.

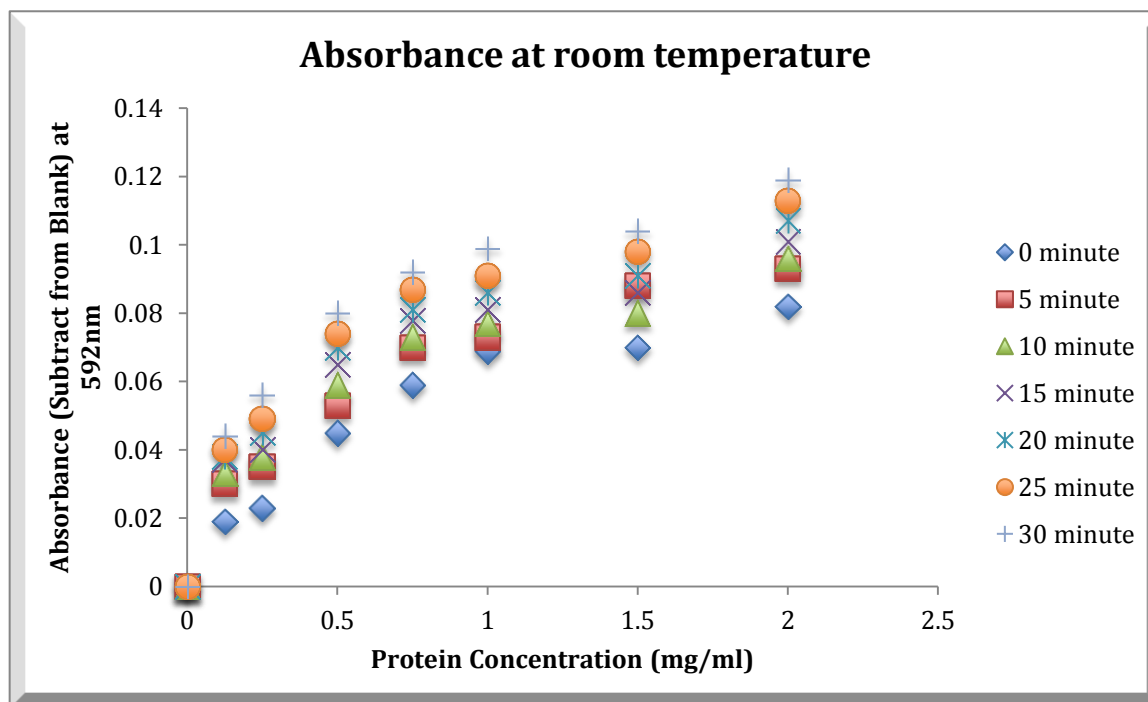


Figure 8 BCA Assay conduct at room temperature at 5-minute interval.

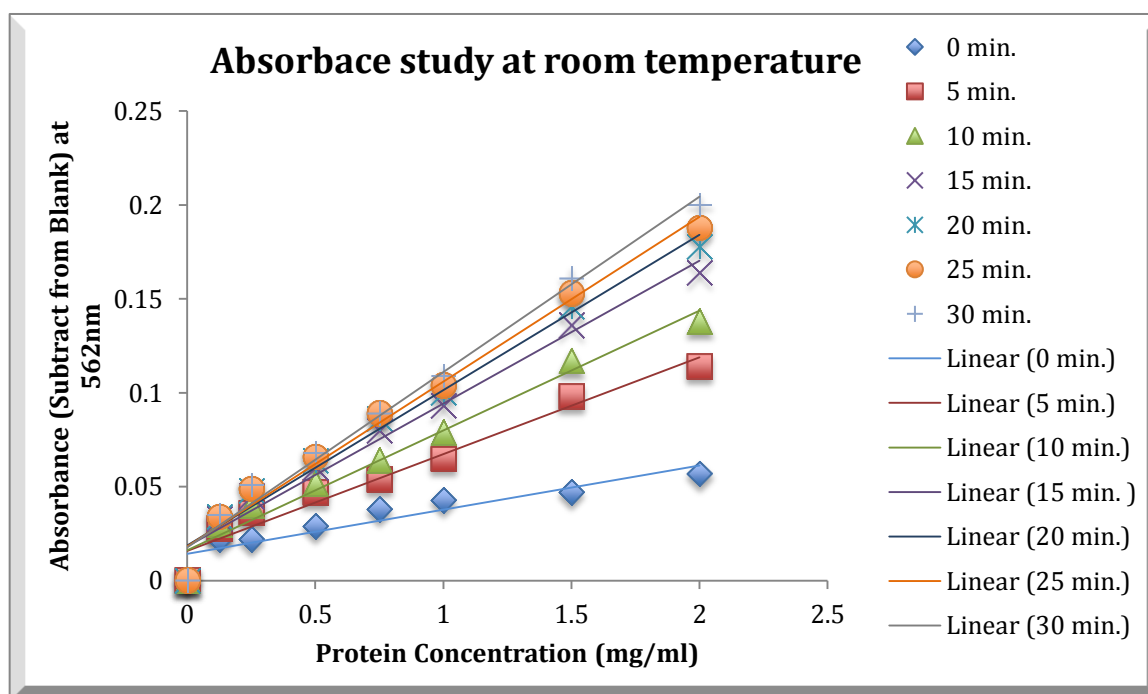


Figure 9 Bradford Assay 5 minutes interval study by incubating the plate.

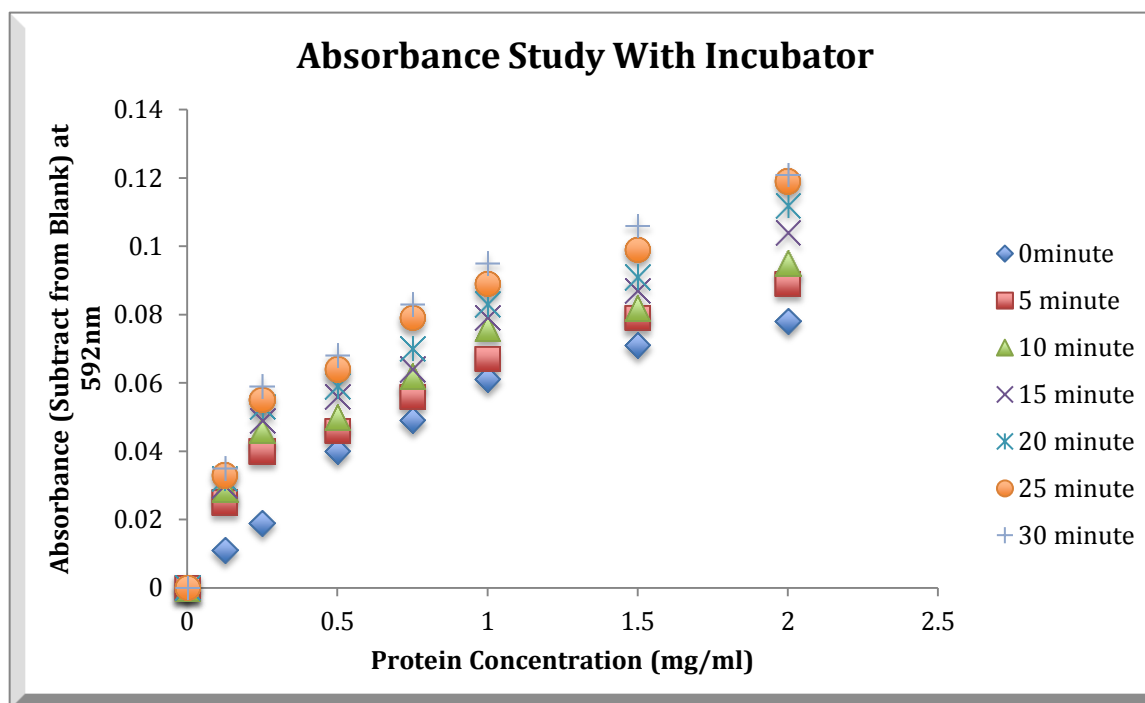


Figure 10 BCA Assay 5 minutes interval study by putting microplate in incubator.

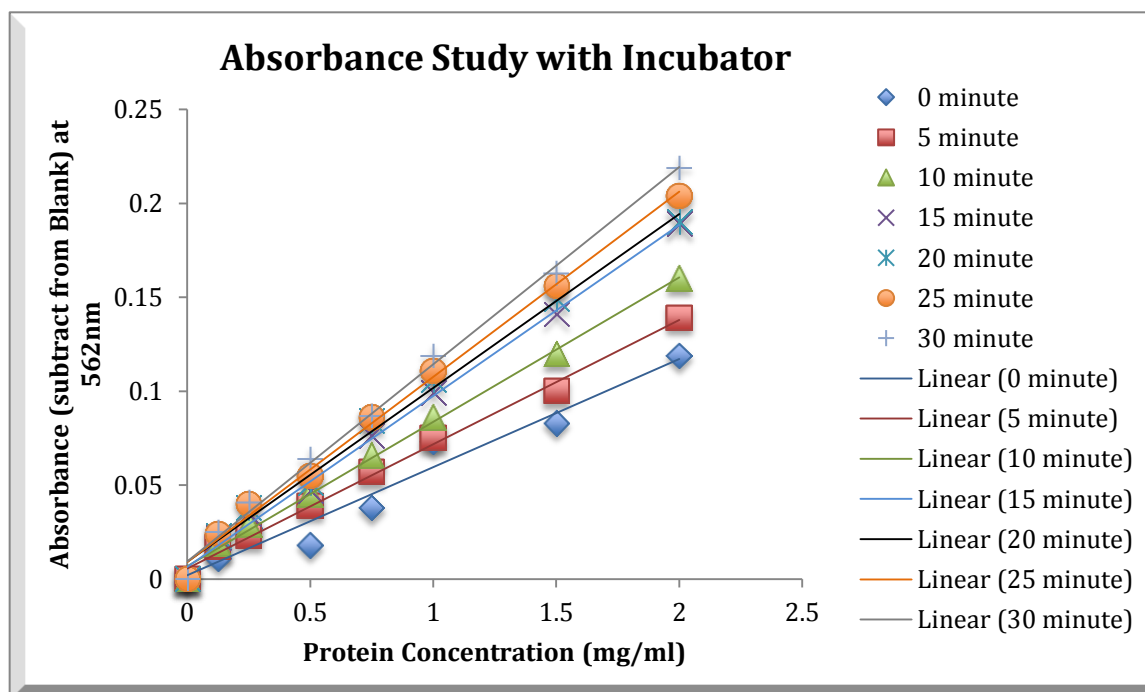


Figure 11 Dilutions for Bradford Assay.



Figure 12 Dilutions for BCA Assay.



Figure 13 Mix the solutions on Parafilm "M".



Figure 14 Transfer the mixtures to the Take3 plate.

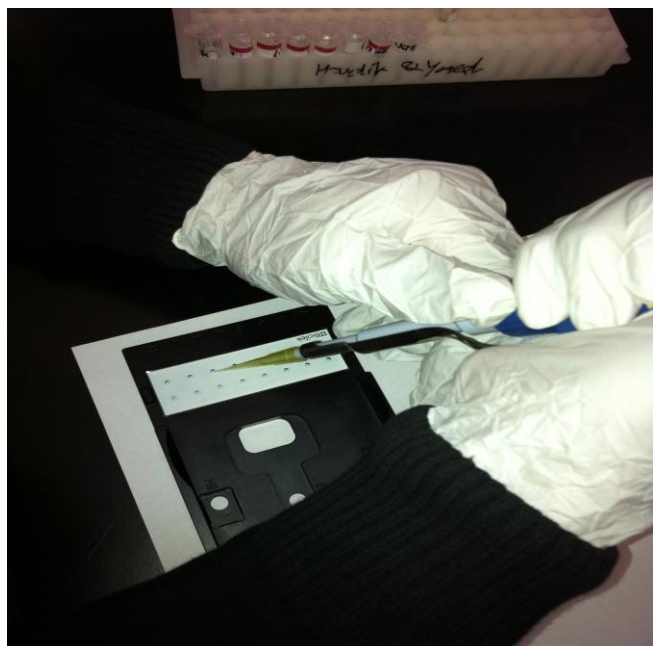


Figure 15 Reagents required for the Microdrop assays.



Figure 16 Instruments used in the Microdrop assays.



Epoch™ by BioTek.



Humidified Incubator.