Protein assay of SpectroArt 200

MATERIAL

- BSA: Albumin, bovine serum (Sigma)
- PBS: BupH™ Phosphate Buffered Saline packs (PIERCE)
- Bradford assay:
  Bio-Rad protein assay (Bio-Rad)
- Lowry assay:
  Na_2CO_3 (Fluka), NaOH (ALDRICH), NaK Tartrate (Fluka), $CuSO_4 \cdot 5H_2O$ (Sigma), Folin solution (Sigma)
- BCA Protein assay kit: (BCA: Bicinchoninic acid)
  Novagen® purchased from Merck
- ddH_20 for solution preparation
- Disposable Cuvettes
- SpectroArt 200 UV-Vis Spectrophotometer (Wealtec Corp., Taipei, Taiwan)

PROCEDURE

1. Bradford Assay:

A. Dilute BSA sample with PBS as following final concentrations:

Concentration (μg/ml)	PBS (μl)	BSA (from 1 mg/ml stock) (μl)
0	800	0
2	798	2
4	796	4
6	794	6
8	792	8
10	790	10

B. Add 200 μl Bio-Rad protein assay dye to each sample and mix well.

C. Pour samples into cuvettes and detect with SpectroArt 200.

D. SpectroArt 200 must be turn on for 10 minutes before using it for warm-up.

E. Press the *"Protein"* on the main menu and press *"Set up"* to modify following

selections.

F. Change the method to the assay that is going to be measured by pressing the *"Method"*. Choose the "*Bradford*" preset method (595 nm).
G. Set up the sample numbers of the standard curve with five different concentrations by pressing *"STD curve"* and key in 5, and set the repetitions of each concentration by pressing *"Blank Rep."*, *"STD Rep."*, and *"Unknown Rep."* to choose and select for three times.
H. Press *"Exit"* to finish the set up and go back to the measuring window.
I. Make sure there is no cuvette in the cuvette chamber and start with *"Dark"* to do the dark blank.
J. Press *"STD"* to start measure.
K. Chose *"µg/ml"* for the concentration that have already made.
L. While there have a dialog with *"Ready for blank sample #1"*, open the sample compartment lid and appropriately insert the cuvette with 0 µg/ml sample into the chamber. Ensure the cuvette does not move around. A steady position of the cuvette is crucial in order to reach good repeatability results.
M. Make sure that the frosted side of the cuvette facing to user and press *"OK"*. Repeat the measurement for three times.
N. Change the sample cuvette to 2 µg/ml. Enter the concentration of sample of "*2*" and press *"OK"* to measure.
O. Repeat the measuring steps until the whole concentration series is finished.
P. After measure the last concentration of 10 µg/ml sample, there will come out with a standard curve on the screen.
Q. If there have unknown sample to measure, press *"Unknown"* to measure the concentration of unknown sample.

2. Lowry assay:

- Prepare the following solutions—
  A: 2% Na_2CO_3 in 0.1 M NaOH solution 50 ml. (The solution should be made freshly)
  B: 1% NaK Tartrate in ddH_2O
  C: 0.5% $CuSO_4 \cdot 5H_2O$ in ddH_2O
  D: Folin solution : ddH_2O = 1:1.
- Mix 250 µl of B solution with 250 µl solution C.
- Add B+C mixture into 12 ml solution A.

- Dilute BSA sample with 0.1 M NaOH as following final concentrations:

Solution Number	Concentration (μg/ml)	Sample volume (μl)	0.1 M NaOH (μl)
1	64	160 μl of BSA stock (1 mg/ml)	140
2	32	150 μl of solution 1	150
3	16	150 μl of solution 2	150
4	8	150 μl of solution 3	150
5	4	150 μl of solution 4	150
6	0	0 μl	150

- Aliquot 150 μl of solution 5 to new tubes to have equal volume with others.
- Add 1 ml A+B+C mixture to each sample.
- React at room temperature (25˚C) for 10 minutes.
- Apply 100 μl solution D into each sample.
- React at room temperature for 30 minutes, then pour samples into cuvettes and detect with SpectroArt 200 (750nm).
- The next procedures are similar with Bradford assay.

3. BCA assay:

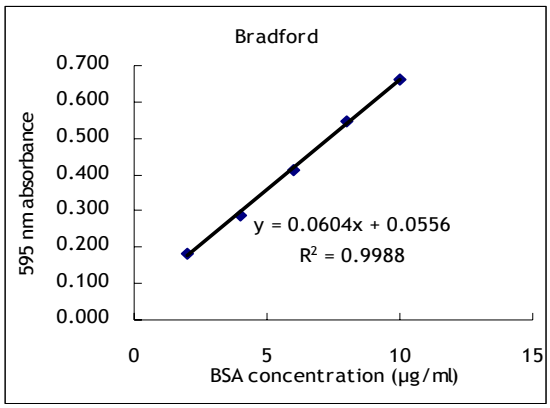
- Mix 7 ml BCA solution with 140 μl 4% cupric sulfate.
- Dilute BSA sample with PBS as following sample concentrations:

Solution number	Concentration (μg/ml)	Sample volume (μl)	PBS (μl)
1	1000	50 μl BSA stock (1 mg/ml)	0
2	500	50 μl of solution 1	50
3	250	50 μl of solution 2	50
4	125	50 μl of solution 3	50
5	25	10 μl of solution 4	40
6	0	0 μl	50

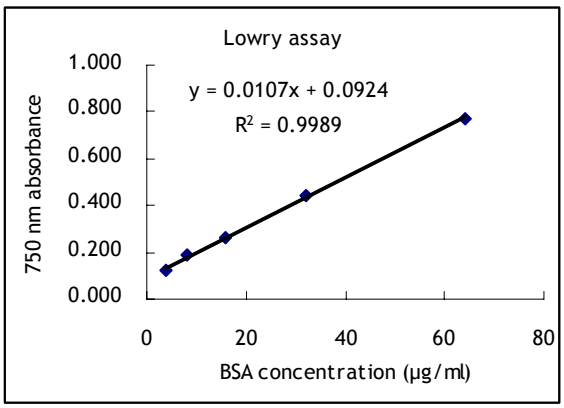
- Aliquot 50 μl of solution 4 to new tubes to have equal volumes with others.
- Add 1 ml BCA working solution into each diluted sample.
- Incubate at 37˚C for 30 minutes.
- Allow the tubes to cool down to room temperature, then pour samples into cuvettes and detect with SpectroArt 200 (562 nm).
- The next procedures are similar with Bradford assay.

RESULTS

(A)



(B)



(C)

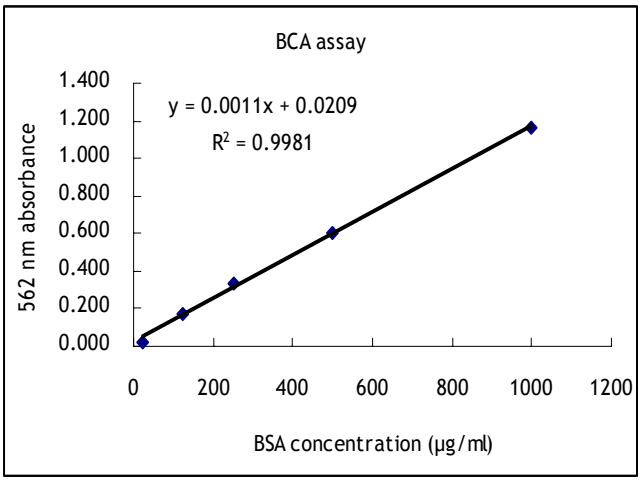


Figure 1. *Linearity of diluted BSA samples. The X-axis is the BSA concentration (µg/ml). The Y-axis is the absorbance for each sample concentration. (A) Standard curve of BSA by Bradford assay. (B) Standard curve of BSA by Lowry assay. (C) Standard curve of BSA by BCA assay.*

- According to Figure 1. Protein assays of SpectroArt 200 can be performed very well with the linearity of R-square values which is around 0.998.
- Different protein assay can be used to determine the sample only within the same condition.

REMARKS

Choosing correct methods for the protein concentration measurement is very important in experimental design. There have many kinds of quantification methods to determine protein concentration, including **Bradford, Lowry, BCA, Biuret, and UV absorbance**…etc. Since there are many interference factors in UV absorption method, it is better to make the standard curve which is for measuring the unknown protein sample at the same time. The principles of recommended methods are as followed:

- **Bradford assay:**
  The major component in the Bio-Rad protein assay solution is Coomassie Brilliant Blue G-250. The Coomassie Blue dye binds to primarily basic and aromatic amino acid residues, especially for arginine and it will change its color from red to blue in perchloric acid after it is bound with proteins. This protein and dye complex will have the highest absorbance at 595 nm. This method is very sensitive, and in order to minimize the sample size and the amount of working solution, it can be also operated on microtiter plate. In the standard procedures, the recommended working concentration for BSA series diluted solution is around 0.2 ~ 0.9 mg/ml (about 3.9 ~ 17.6 μg/ml final concentration). In the microassay procedures, the recommended working concentration for BSA series diluted solution is around 1.2~ 25 μg/ml (about 0.96 ~ 20 μg/ml final concentration).
- **Lowry assay:**
  Lowry assay is derived from Biuret method. The Biuret solution is an alkaline copper sulfate solution. Copper ion will combine with protein and it can be reduced from divalence to monovalence copper, and form a complex with pink to purple blue color. The color of the complex depends on the size of the protein under basic surrounding. After react with protein solution, copper ion can further reduce the phosphomolybdic phosphor- tungstate in Folin regent and form a blue color solution with aromatic amino acid residues of the protein which has the highest absorbance at 750 nm. This kind of reaction is not very stable. The color component will decompose very fast after the reaction. Lowry assay is mainly interfered by ammonia sulfate, Tris, ammonia, thiol reagent, magnesium ion, potassium ion, EDTA, carbon hydrate, and glycerol. The sensitivity of Lowry assay is around 0.1 mg. The recommended working BSA series diluted solution concentration is around 1~1500 μg/ml (about 0.12 ~ 180 μg/ml final concentration).

- BCA assay:
  The principle of BCA method is similar to Lowry assay, but it is using BCA solution instead of Folin regent. Monovalence copper in BCA solution will form a dark purple color compound with the highest absorbance at 562 nm. This component can stand longer than those in Lowry assay. The kit can be used to determine working protein concentration in the range from 20 to 2000 μg protein per milliliter either for standard assay or microassay size. The interferences of BCA assay are reducing sugars and EDTA.

Every assay has its own detect limitation and dye reaction site. Also, the spectrophotometer has its own convincible absorbance range, normally located in 0.2~1.2 absorbance units. The assays here all have commercial kit now provided by many different famous bioscience companies. Operators should read all the instructions before processing experiment. The best way to determine the concentration of protein is to choose suitable method which according to the composition of amino acids of the protein that is going to be detected, the solution content that is working on, and in what range the protein concentration will fall in. While operating the assay, people should avoid any interference in the working solution as possible. It is also very important to have an adequate sample concentration within the BSA dilution series and suitable absorbance within the convincible absorbance range. To prevent the decomposition of the coloring compound and affect the absorbance result, make sure to measure the absorbance of the sample as soon as possible.

According to the result that had been repeat tested, the SpectroArt 200 performance is very good for testing with all kinds of protein assay which R-square values is over 0.998. The thoughtful preset options program of the operation system which designed for detecting various protein assays. It can provide precise absorbance wavelength and prevent wrong operation for wavelength detections. It also presents with good data reproducibility and gives excellent reliable data for routine use.

Bibliography

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