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Bradford protein assay – Protein concentration measurement (A590/A450 improved linearity)

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ABSTRACT

This protocol describes how to measure the concentration of total protein in a sample performing the Bradford's assay using microtiter plates. Procedures are slightly modifications based on the method described in (1) and the linearization procedure described in (2) and (3).

(1) Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

(2) Zor & Selinger, Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies, Anal. Biochem. 236 (1996) 302–308.

(3) Ernst & Zor, Linearization of the Bradford protein assay, J. Vis. Exp. 38 (2010) 1918.

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PROTOCOL CITATION

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KEYWORDS

Protein, Protein determination, Colorimetric assay, Bradford, Microtiter plate, Microplate

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MATERIALS TEXT

MATERIALS

Whatman #1 paper **Contributed by users Catalog #1001150**

Bio-Rad Protein Assay Dye Reagent Concentrate **BIO-RAD Catalog #5000006**

Bovine Serum Albumin lyophilized powder essentially fatty acid free and essentially globulin free **Sigma Aldrich Catalog #A0281**

Ultrapure water (Type 1) **Contributed by users**

Corning® 96 well NBS™ Microplate **Sigma Aldrich Catalog #CLS3651**

UV-transparent cuvettes **BIO-RAD Catalog #1702511**

Coomassie Brilliant Blue G **Sigma Aldrich Catalog #B0770**

Ethanol **Fisher Scientific Catalog #10538071**

Phosphoric acid solution **Sigma Aldrich Catalog #W290017**

SpectraMax M3 Multi-Mode Microplate Reader
Microplate Reader

Molecular Devices 8002482

Multi-mode microplate readers (Absorbance, Fluorescence (top/bottom read), and Luminescence (top/bottom read)). Ranges: Abs, 200–1000 nm; FL, 250–850 nm; Lumi, 250–850 nm. Light source: Xenon flashlamp. Detector: Silicon photodiode, Photomutiplier tube. Readtypes: Endpoint, Kinetic, Spectrum scan, and Well scan.

Bradford's protein reagent preparation

1 **Prepare a solution containing 0.01% (w/v) Coomassie Brilliant Blue G-250 (e.g., B0770, Sigma-Aldrich), 4.7% (v/v) ethanol, and 8.5% (v/v) phosphoric acid as described in the next steps.**

2 **Weight 100 mg of Coomassie Brilliant Blue G-250 (e.g., B0770, Sigma-Aldrich) and dilute it in 50 mL of ethanol using a magnetic stirrer at room temperature.**

3 **In a fume hood, slowly add 100 mL of 85% (w/v) phosphoric acid (e.g., W290017, Sigma-Aldrich) to the previous solution and homogenize.**

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4 **Dilute the resulting solution to a final volume of 1,000 mL with distilled water[1].** Filter through a Whatman No. 1 paper (or equivalent). Store at room temperature protected from light.

[1] Alternatively, you can use commercially available ready-to-use reagents (e.g., 5000006, Bio-Rad).

Protein standard solutions preparation

5 **Prepare a ~4 mg/mL bovine serum albumin (BSA, e.g., A0281, Sigma-Aldrich) stock solution in PBS[2].**

Example: Dilute 6 mg in a final volume of 1.5 mL.

[2] If compatible[3], use the same solvent/buffer in which the sample to be analyzed was prepared to make the BSA standard solutions.

[3] Check buffer components incompatibility with the Bradford reagent. There are several available online. For example: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6852.pdf and https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/b6916bul.pdf.

6 ⚠

Check the BSA concentration in the stock solution. Read it at 280 nm in an UV-transparent cuvette and use the equation below to calculate the actual BSA concentration.

$$BSA\ (mg/mL) = \left(\frac{Abs_{280} \times 10}{6.6}\right) \times dilution$$

Example: If you diluted 50 µL of the BSA stock solution in a final volume of 1,000 µL and it resulted in a absorbance of 0.130, you have:

$$BSA\ (mg/mL) = \left(\frac{0.130 \times 10}{6.6}\right) \times 20$$

$$BSA\ (mg/mL) = 3.94$$

7 **Prepare several BSA solutions at concentrations from 0.025 to 0.80 mg/mL[4] using PBS (or the relevant buffer/solvent).**

Example: If your stock solution is 3.94 mg/mL, first prepare a 0.8 mg/mL (100 µL to a final volume of 492.5 µL) and then dilute it serially to 0.40, 0.20, 0.10, 0.05 and 0.025 mg/mL (e.g., 100 µL of the previous solution + 100 µL of PBS).

[4] Under the conditions described in this protocol, the the ratio of absorbances at 590 nm over 450 nm is linear within the 0.025–1.00 mg/mL BSA range (see figure below).

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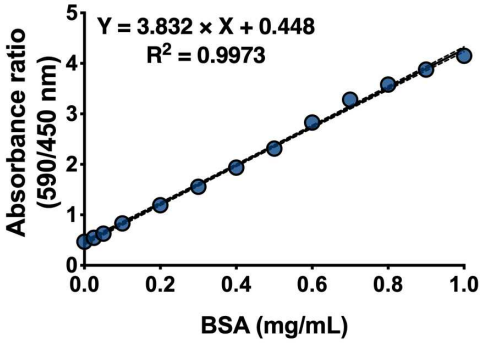

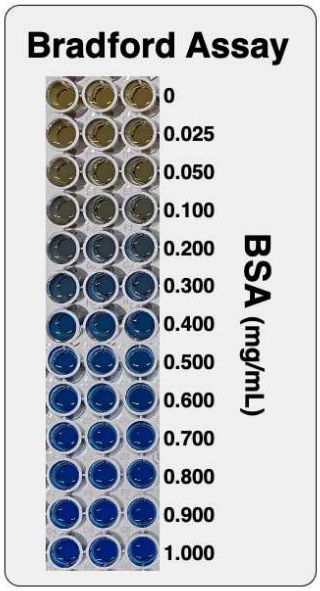

Sample preparation

8 **Dilute samples in appropriate buffer (e.g., PBS; see section/step 5) to achieve an expected concentration[5] that lies within the concentration range of the standard curve (0.025–0.8 mg/mL; see section/step 7).**

Example: Considering that rat liver contains ~100 mg protein/g wet weight, if you have a rat liver extract/homogenate that was prepared diluting the tissue sample 1:20 (resulting in ~5 mg protein/mL), you need to further dilute by a factor of least 15 (total dilution 1:300, resulting in ~0.333 mg/mL) before proceeding with the assay.

[5]If an estimate of protein concentration for your tissue/cell sample is not available, test several dilutions.

Assay

9 **Add 260 µL of water to at least three wells of a 96-well microtiter plate.**

These will be the microplate reader blanks (also known as 'auto zero' or 'reference wells').

10 **Add 10 µL of each BSA standard solution to at least three wells of a 96-well microtiter plate.**

Remember to prepare a zero/blank standard, which is the buffer used to prepare the standards but with no BSA.

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11 **Add 10 µL of each sample to at least three wells of a 96-well microtiter plate.**

Ideally, you should use several dilutions of the sample and check whether there is a linear response between signal (the ratio absorbance at 590 nm over absorbance at 450 nm) and amount of sample. One convenient assay of doing it is to pipet several volumes (e.g., 1, 2, 5, 10 µL) and add it up to 10 µL with the appropriate buffer. This also maximizes the chance of getting at least one dilution of the sample within the dynamic range of the standard curve for unknown samples.

Example:

- Dilution 1 – 1 µL sample + 9 µL buffer;
- Dilution 2 – 2 µL sample + 8 µL buffer;
- Dilution 3 – 5 µL sample + 5 µL buffer;
- Dilution 4 – 10 µL sample.

12 **Add 250 µL of Bradford's protein reagent to each well containing BSA standards or samples.**

If available, use a positive displacement pipette device (e.g., Multipette M4, Eppendorf) to avoid bubbles.

Do not add Bradford's protein reagent to wells containing 260 µL water. These are used just as blanks for the microplate reader.

13 **Incubate the 96-well microtiter plate at room temperature for 5 min.**

14 **Read the microplate at 590 nm and 450 nm.**

Read before 1 h of incubation. At high protein concentrations, precipitation begins after 10–15 minutes.

Note: In case a microplate reader is not available, a picture taken with a smartphone camera can be used to acquire data and accurately calculate protein levels in samples (see the protocol '"Bradford protein assay – Protein concentration measurement using RGB data")

Calculation

15

Calculate the average of the ratio between absorbances (A590/A450) for each BSA standard and samples using the absorbance values of the triplicates.

Example data:

BSA	Absorbance at 590 nm				Absorbance at 450 nm				Ratio A590/A450			
mg/mL	Well 1	Well 2	Well 3	**Average**	Well 1	Well 2	Well 3	**Average**	Well 1	Well 2	Well 3	**Average**
0.00	0.379	0.379	0.378	0.379	0.811	0.810	0.804	0.808	0.467	0.468	0.471	0.469
0.025	0.437	0.427	0.433	0.432	0.793	0.793	0.793	0.793	0.551	0.538	0.546	0.545
0.05	0.495	0.481	0.481	0.486	0.772	0.781	0.763	0.772	0.642	0.616	0.630	0.629
0.10	0.583	0.600	0.604	0.596	0.715	0.718	0.720	0.718	0.816	0.835	0.839	0.830
0.20	0.755	0.787	0.785	0.776	0.661	0.643	0.650	0.651	1.143	1.224	1.207	1.191
0.40	1.041	1.004	1.037	1.027	0.547	0.517	0.527	0.531	1.902	1.940	1.967	1.936
0.80	1.454	1.479	1.481	1.471	0.404	0.412	0.417	0.411	3.596	3.590	3.554	3.580

BSA standard solutions.

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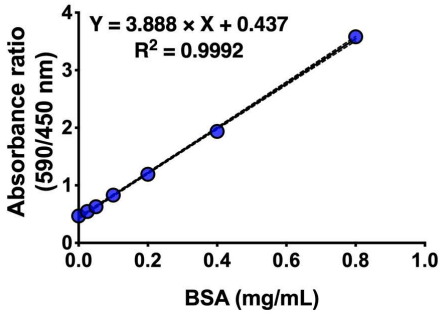
Sample	Ratio A590/A450			
	Well 1	Well 2	Well 3	**Average**
Rat liver homogenate (1:500)	0.870	0.879	0.874	0.874

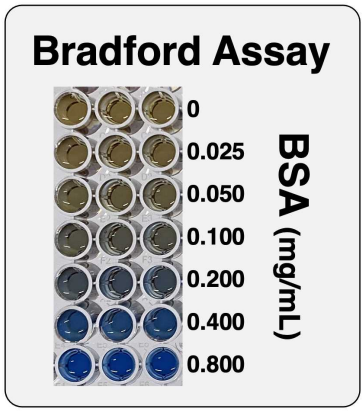
Sample example: 5 µL of rat liver homogenate (1:500, w/v) + 5 µL PBS.

16 **Build a standard curve**.

Plot the average A590/A450 ratio on the Y- axis *versus* BSA concentration in mg/mL on the X-axis. Calculate a linear regression (Y = a × X + b; e.g., 'add a linear trendline' in Microsoft Excel).

Example:





17 **Calculate protein concentration in samples using the equation generated in the previous step.**

Interpolate unknown protein concentration from the standard curve and multiply by all dilutions.

$$Protein\ (mg/mL) = \left(\frac{((A590/A450) - b)}{a}\right) \times Dilution\ 1 \times Dilution\ 2$$

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Example:

For this example (see step 15), the dilution factors are *500* (a rat liver homogenate was prepared at 1:500, w/v) and *2* (5 µL were added to the microplate and added up to 10 µL with buffer).

$$Protein\ (mg/mL) = \left(\frac{(0.874 - 0.437)}{3.888}\right) \times 500 \times 2$$

$$Protein\ (mg/mL) = 112.5$$

For tissues, the final result is often expressed as mg protein per gram of wet weight (mg/gww) assuming 1 g/mL. Thus, this rat liver sample used as example would have 112.5 mg/gww.

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