Assay kits

Bradford Assay Kit

Bradford Protein Assay Kit

Protocol

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IMPORTANT NOTES – Before you begin

- ✓ OZ Biosciences developed this **Bradford Protein Assay Kit** to accurately quantify the concentration of proteins in solution and to normalize your transfection experiments since it is functional with several reporter gene assay kits such as the β-galactosidase, luciferase assay kits...
- ✓ The Bradford Protein Assay Kit is a straightforward and rapid procedure.
- ✓ The Bradford Protein Assay Kit is based on the binding of Coomassie Brilliant Blue G-250 dye to the proteins and particularly basic and aromatic amino acids residues. The dye exists in three forms: cationic (red), neutral (green) and anionic (blue). Under acidic conditions, the dye is predominantly in the protonated cationic form (red, Amax = 470 nm). When the dye binds to proteins, it is converted to a stable unprotonated form (blue, Amax = 595 nm). It is this blue unprotonated form that is detected at 595 nm to quantify the concentration of proteins.

- ✓ The improved and optimized 1X Bradford reagent buffer allows superior linearity of response at low and high protein concentrations and determination of protein amount in the presence of detergent (< 0.1%).
- ✓ Standards are provided in a 145mM NaCl, 0.05% NaN_3 solution.

Standards (µg/mL)	Corresponding color
1500	Red
1000	Orange
750	Pink
500	Purple
250	Light Purple
100	Green
50	White

CAUTION: **Phosphoric acid is a corrosive liquid.**

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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Package content	BA00100: one bottle of 1X Bradford reagent (500 mL) and 2 x 7 vials of pre-diluted BSA standard BA00050: one bottle of 1X Bradford reagent (500 mL) BA00070: 2 x 7 vials of pre-diluted BSA standard Number of protein assays given for a concentration range from 0.5 µg/mL to 50 µg/mL: - Number of assays (96-well plate): 5000 - Number of assays (1mL cuvettes): 1000
Shipping conditions	The kit is shipped at Room Temperature.
Storage conditions	Upon receipt and for long-term use, store all reagents at + 4°C. The Bradford Protein Assay Kit is stable for at least one year at the recommended storage temperature.
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

General Considerations

- For your convenience, both the 1X Bradford reagent and the protein assay standard (two sets of 7 pre-diluted concentrations) are ready to use.

- ***Before each use***, let warm the 1X Bradford reagent to ambient temperature and turn upside down the bottle a few times. It is preferable to maintain a constant temperature during the assay since absorbance measurement with the Bradford reagent is temperature dependent.

- Protein stain with the Bradford method is highly protein dependent. Consequently, the best protein to use as a ***standard*** is the protein being assayed. Nevertheless, in the absence of such standard, another protein can be used. The two most common **protein** standards used for protein assays are Bovine Serum Albumin and Bovine Gamma-Globulin. A convenient pre-diluted BSA standard curve is included in this kit that is linear for high and low protein doses. In order to make accurate protein concentration measurements, the protein assayed needs to be in the linear region of the standard curve. Thus, the dilution of the standard curve used need to be adjusted accordingly.

- Some ***chemicals*** interacting with protein can interfere with the assay. Interference from these compounds is due to their ability to shift the equilibrium levels of the dye among the free color species, by direct binding or by shifting the pH. The detergents used to prepare cell lysates, flavonoids and basic buffers are known to alter this protein assay. However, the **Bradford Protein Assay Kit** is compatible with low amount of these chemicals as indicated in the Table 1. See the standard cell lysate protocol developed below for cell lysates or protein samples containing some limited amount of detergents.

Table 1: Concentrations of some common reagents compatible with the Bradford protein assay.

Acetone 10%	Guanidine-HCl, 2M	Sodium acetate pH4.8, 0.2M
Acetonitrile 10%	HCl, 0.1M	Sodium azide, 0.5%
Ammonium sulfate, 1M	HEPES, 0.1M	Sodium bicarbonate, 0.2M
Ampholytes, 0.5%	Imidazole, 0.2M	Sodium carbonate, 0.1M
ASB-14, 0.025%	Magnesium chloride, 1M	Sodium chloride, 2.5M
Ascorbic Acid, 50mM	MES, 0.1M	Sodium citrate, pH4.8 or 6.4, 0.2M
Bis-Tris, pH6.5, 0.2M	Methanol, 10%	Sodium hydroxide, 0.1M
β-mercaptoethanol, 1M	MOPS, 0.1M	Sodium phosphate, 0.5M
Calcium chloride, 40mM	NAD, 2mM	Sucrose 10%
CHAPS, 10%	NP-40, 0.25%	TBP, 5mM
CHAPSO, 10%	Octyl β-glucoside, 0.5%	TCEP, 20mM
Deoxycholic acid, 0.2%	Octyl β-thioglucopyranoside, 1%	Thio-urea, 1M
DMSO, 5%	Phenol Red, 0.5 mg/mL	Tricine, pH8, 50mM
DTE, 10mM	PIPES, 0.2M	Triethanolamine, pH7.8, 50mM
DTT, 5mM	PMSF, 2mM	Tris, 1M
EDTA/EGTA, 0.2M	Potassium chloride, 2M	Tris-glycine
Ethanol, 10%	Potassium phosphate, 0.5M	Triton X-100, 0.05%
Glucose, 20%	RIPA lysis buffer, 1/40 dilution	Tween 20, 0.01%
Glycerol, 5%	SB 3-10, 0.1%	Urea 4M
Glycine, 0.1M	SDS, 0.025%	

- ***Wavelength.*** You can use any wavelength between 580 nm and 610 nm. However, the maximum sensitivity of the assay is reached at 595 nm.

- ***Molecular weight***. The lower limit of detection for the Bradford method is 3,000-5,000 Da.

- ***Data analysis***. Subtract the average blank value from the standard and the unknown sample values. Create a standard curve by plotting the 595 nm values (y-axis) versus the concentration of protein in µg/mL (x-axis). Determine the unknown sample concentration using that curve. If the samples assayed were diluted, adjust the final concentration by multiplying by the dilution factor.

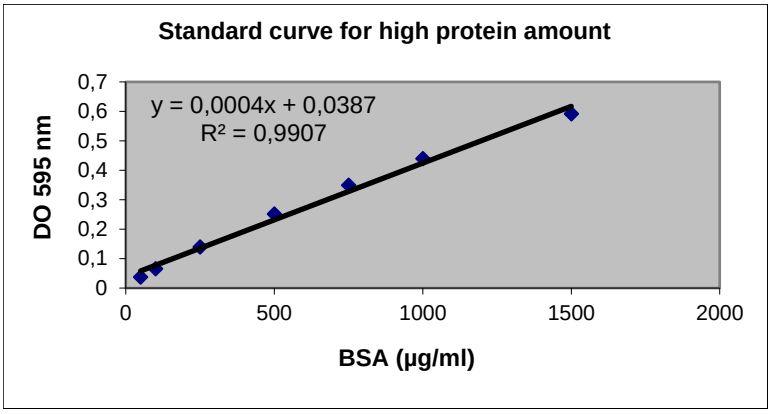
We have developed three specific protocols for different microassays: high protein concentration, low protein concentration and cell lysates. In addition, these protocols can be adapted to macroassay by just multiplying the reagent quantity by five or six. Please refer to the appropriate procedure for your particular application.

1. Standard 96-well plate assay

High protein amount microplate assay

This assay is designed to measure protein concentration in solution from 50 to 1500 µg/mL.

1) **BSA standard curve**: Add 10 µL of each pre-diluted BSA standard vials in 7 different wells of a 96-well plate. In this way, you will have the following standard curve: 1500, 1000, 750, 500, 250, 100, 50 µg/mL. For the negative control (blank, 0 µg/mL) add 10 µl of water, buffer or saline solution (PBS, HBS, etc…) to a well.



2) Put 10 µL of your protein(s) in an empty well. Serial dilution of the protein to be assayed can also be performed.
3) Add 140 µL / well of the Bradford reagent and mix the solution by pipetting or with a microplate mixer.
4) Incubate 5 min at room temperature. Do not incubate more than 1 hour at room temperature.
5) Read the absorbance at 595 nm with a microplate reader.

Low protein amount microplate assay

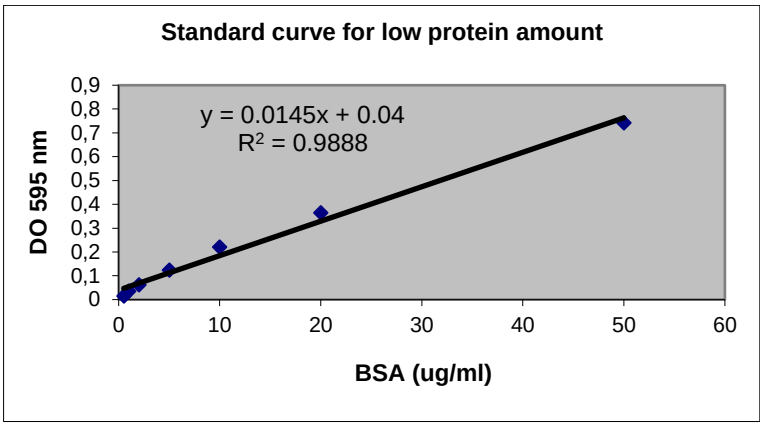
This assay is designed to measure protein concentration in solution from 0.5 to 50 µg/mL.

1) Prepare a serial dilution of the protein standard (BSA) as suggested in the table 2.

Table 2: Preparation of BSA standard dilution curve for low protein amount detection.

Protein standard (BSA) volume	Buffer added	µg of BSA / mL
8 µL of the 1500 µg/mL stock solution (red color)	232 µL	50
100 µL of the 50 µg/mL solution	150 µL	20
125 µL of the 20 µg/mL solution	125 µL	10
125 µL of the 10 µg/mL solution	125 µL	5
125 µL of the 5 µg/mL solution	125 µL	2.5
125 µL of the 2.5 µg/mL solution	125 µL	1.25
125 µL of the 1.25 µg/mL solution	125 µL	0.625

2) Add 100 µL of each standard dilution vials in 7 different wells of a 96-well plate. In this way you will have the following standard curve: 50, 20, 10, 5, 2, 1, 0.5 µg/mL. For the negative control (blank) add 100 µl of water, buffer or saline solution (PBS, HBS, etc…) to a well.



3) Put 100 µL of your protein(s) in an empty well. Serial dilution of the protein to be assayed can also be performed.

4) Add 100 µL / well of the Bradford reagent and mix the solution by pipetting or with a microplate mixer.

5) Incubate 5 min at room temperature. Do not incubate more than 1 hour at room temperature.

6) Read the absorbance at 595 nm with a microplate reader.

Cell lysate microplate assay

This assay is intended for measuring the protein concentration in whole cell lysates. The calculated concentration corresponds to the total amount of cellular protein. This assay can be used for your transfection experiments normalization.

1) Aspirate the growth medium from your cell culture dish, for instance, 24-72 h post-transfection.

2) Wash your cells twice with PBS as serum containing proteins interfere highly with the assay.

3) Lyse your cells with a lysis buffer. Refer to table 3 for the volume of lysis buffer to use.
Suggested lysis buffer composition: 250mM Tris pH7.4 and 0.1% Triton X-100

Table 3: Volume of Lysis Buffer in function of culture dish.

Type of culture dish	Volume of Lysis buffer (µL/well)
96-well plate*	50*
24-well plate	250
12-well plate	500
6-well plate	1000
60 mm dish	2500
100 mm dish	5000

* For 96-well plate transfection experiments where protein and reporter gene assays will be used to normalize the results, lysed the cells in 50 µL of lysis buffer and add 50 µL of dilution buffer. Then, use 50 µL for protein assay and 50 µL for reporter gene assay. One of the two assays can be performed directly into the 96-well plate used for cell culture. If you are using 96-well plate format for your cell cultures, you can lyse the cells in 25 µL of lysis buffer, then add 25 µL of dilution buffer and perform the protein assay kit directly into the same plate.

4) Incubate your cell lysates 10-15 min at room temperature. A fast freeze/thaw cycle can also be done to achieve a good lysis.

5) Add 1 volume of dilution buffer to your cell lysates in order to reduce the amount of detergent (0.05 % Triton X-100 final concentration). Cell lysates can be centrifuged 2-3 min to pellet the insoluble material. Suggested dilution buffer: 250mM Tris pH7.4

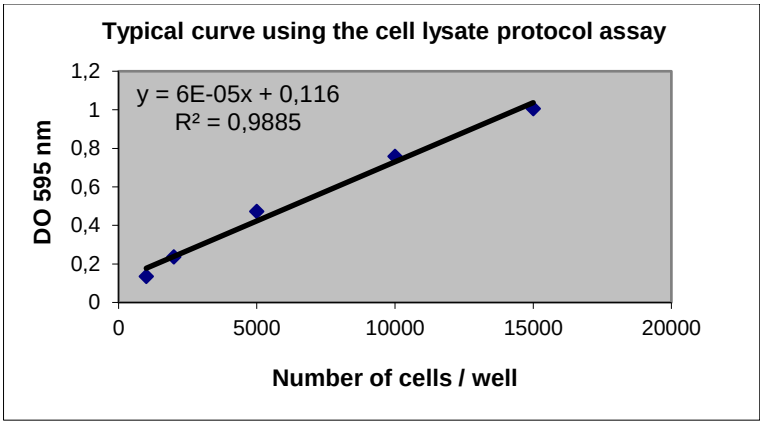
6) Transfer 50 µL of each diluted cell lysates to empty wells of a 96-well plate (flat bottom). The remaining cell lysate can be used to monitor reporter gene assay.

7) Prepare the BSA standard curve as follow.

Table 4: Preparation of BSA standard dilution curve for cell lysate protein assay.

Protein standard (BSA) volume	Lysis buffer*	Dilution buffer*	µg of BSA / mL
10 µL of the 1500 µg/mL stock solution (red cap)	70 µL	70 µL	100
60 µL of the 100 µg/mL solution	30 µL	30 µL	50
60 µL of the 50 µg/mL solution	30 µL	30 µL	25
60 µL of the 25 µg/mL solution	30 µL	30 µL	12.5
60 µL of the 12.5 µg/mL solution	30 µL	30 µL	6.25
60 µL of the 6.25 µg/mL solution	30 µL	30 µL	3.12
60 µL of the 3.12 µg/mL solution	30 µL	30 µL	1.56
Blank	30 µL	30 µL	0

* Instead of using a lysis buffer and a dilution buffer you can directly prepare and use 0.05 % Triton X-100 containing buffer. Add 60 µL of that buffer instead of 30 µL of lysis buffer plus 30 µL of dilution buffer.



8) Transfer 50 µL of each BSA standard dilution to empty wells of the 96-well plate.

9) Add 150 µL of the 1X Bradford reagent in each well and mix the solution by pipetting or with a microplate mixer

10) Incubate 5 min at room temperature. Do not incubate more than 1 hour at room temperature.

11) Read the absorbance at 595 nm with a microplate reader.

2. Macro Protein assay

The Bradford Protein Assay can also be performed in 1 mL cuvette assay. The procedure is as simple:

For high protein amount assay, just multiply by six the amounts indicated for microplate procedures.
For Low protein amount assay, just multiply by five the amounts indicated for microplate procedures.

Appendix

Guidelines for preparing your own standard curve with 2 mg/mL BSA or Gamma-Globulin stock solution.

High protein amount standard curve:

Protein standard (BSA) volume	Buffer added*	µg of BSA / mL
30 µL of the stock solution	10 µL	1500
20 µL of the stock solution	20 µL	1000
20 µL of the 1500 µg/mL solution	20 µL	750
20 µL of the 1000 µg/mL solution	20 µL	500
20 µL of the 500 µg/mL solution	20 µL	250
20 µL of the 250 µg/mL solution	30 µL	100
20 µL of the 100 µg/mL solution	20 µL	50

Low protein amount standard curve:

Protein standard (BSA) volume	Buffer added*	µg of BSA / mL
10 µL of the stock solution	390 µL	50
4 µL of the stock solution	396 µL	20
200 µL of the 20 µg/mL solution	200 µL	10
200 µL of the 10 µg/mL solution	200 µL	5
200 µL of the 5 µg/mL solution	200 µL	2.5
200 µL of the 2.5 µg/mL solution	200 µL	1.25
200 µL of the 1.25 µg/mL solution	200 µL	0.625

NOTES

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Additional products for protein Dosage

- **FluoProdige Assay Kit** for protein and peptide quantification
- **BCA-PAK Protein Assay Kit** for colorimetric detection and quantification of total protein content

Purchaser Notification

Limited License
The purchase of the Bradford Protein Assay Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Bradford Protein Assay Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Bradford Protein Assay Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a Bradford Protein Assay Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Bradford Protein Assay Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

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