Protein Quantification Kit (Bradford Assay)

Cat #: orb1147875(manual)

Size: 500 T/2000 T/5000 T

Micro NADH Oxidase (NOX) Activity Assay Kit	
Cat #: orb1147875	**Lot #:** Refer to product label
protein concentration range: 50-1000 μg/mL	
Storage: Stable for at least 12 months at 4℃ from date of shipment. The Bradford Reagent are stable at 4℃. The BSA Standard should be aliquoted after the first thaw and stored at -20℃.	

Assay Principle

Biorbyt Protein Quantification Kit (Bradford Assay) provides a simple and rapid procedure for determining the concentration of protein in samples. The method utilizes an improved Coomassie blue G-250 reagent which forms a blue complex in the presence of protein. When coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue. The intensity of the blue complex is proportional to the amount of protein in the sample. The Bradford Protein Assay is suitable for measuring protein concentration in the range of 50-1000 μg/mL (1-20 μg protein).

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	500 T	2000 T	5000 T	
Bradford Reagent (2×)	50 mL	200 mL	500 mL	4℃
BSA Standard (10 mg/mL)	1 mL	4 mL	10 mL	-20℃

Materials Required but Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader or spectrophotometer

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- 96 well plate or Test-tube
- Orbital shaker

Reagent Preparation

Note: Bring all reagents to room temperature before use. If crystals have formed in the Bradford Reagent Concentrates, warm them gently until they completely dissolved.

Bradford Working Solution: Prepare working solution by diluting the 2 × Bradford Reagent with distilled water. The total volume made will depend upon the number of samples and standards to be quantitated. Each sample and standard will require 200 µ L of working solution.

Sample Preparation

Sample Solution: Dilute samples to fall within 0.05- 1 mg/mL range.

Assay Procedure

A. Microplate Procedure

1 . Standard Solution Preparation. Label 8 tubes 0-7. Dilute the BSA Standard to 1 mg/mL working Solution. Ideally, use the same buffer contained in your samples. Then prepare serial dilutions as follows.

Tube	0	1	2	3	4	5	6	7
BSA working Solution (µL)	0	5	10	20	40	60	80	100
Buffer (µL)	100	95	90	80	60	40	20	0
Concentration (µg/mL)	0	50	100	200	400	600	800	1000

2. Pipette 20 µL Standards or samples into duplicate wells in a clear bottom 96 well plate.

3. Add 200 µL of Bradford Working Solution into each well that contains the standard or samples.

4. Shake gently to mix. Incubate for 5 min

5. Measure OD at 595 nm. The signal is stable for at least 30 min.

B. Test-tube Procedure

According to the specification of the Test-tube, as in the above method, the volume of each solution may be appropriately increased in proportion.

Data Analysis

• Subtract the blank OD (zero standard) from all standard and sample OD values. Plot the corrected OD against standard protein concentrations. Use the standard curve to determine the sample protein concentration.

• Alternatively, the equation for the best line fitting the standards can be used to determine the protein concentration of your samples.

• If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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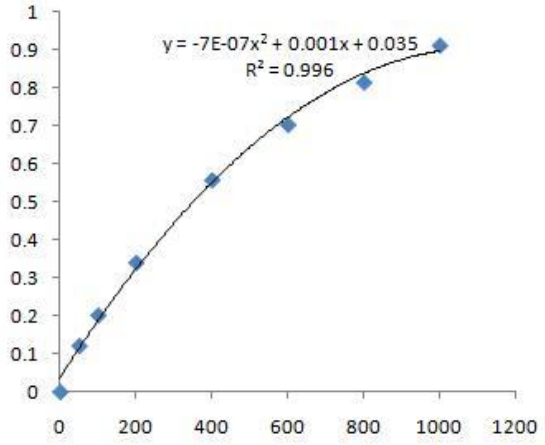
Typical Data

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Precautions

1. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

2. Dye-dye aggregates can form over several hours in stored reagent while dye-protein-dye aggregates form more quickly. Fortunately, gentle mixing completely disperses the dye-dye aggregates. Therefore, it is good practice to mix the Bradford Reagent before pipetting and to mix each tube or plate immediately before measuring absorbances.

3. Certain substances are known to interfere with bradford assay including most ionic and nonionic detergents, which reduce color development and can cause precipitation of the assay reagent.

4. Certain substances interfere to a lesser extent with protein estimation using the bradford assay, and these have only minor (tolerable) effects below a certain concentration in the original sample. Such as, EDTA, Glucose, DTT, 2-Mercaptoethanol and so on.

5. When assaying protein in solutions containing reducing agents, best results are obtained by adding the same amount of reducer to the wells containing the protein standard.

6. If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Recommended Products

Catalog No.	Product Name
orb1147876	Protein Quantification Kit (BCA Assay)
orb1173368	Colorcode Prestained Protein Marker (10- 180 kDa)
orb1173367	Colorcode Prestained Protein Marker (15- 130 kDa)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.