-Proteostain-
Protein Quantification Kit-Rapid (500 tests, 2500 tests)
Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/pq01.pdf

General Information

For protein concentration determination, several methods are available, such as Lowry method, bicinchoninate method (BCA method), Biuret method, and Bradford method. Coomassie Brilliant Blue G (Fig. 1) has been utilized for quick and sensitive protein detection known as the Bradford method. Coomassie Brilliant Blue G interacts with protein and stains blue under acidic conditions. The maximum change in absorbance by interaction with proteins is at 595 nm (Fig. 2). The staining reaction completes within 1 minute and the color is stable for 30 minutes. Therefore, protein concentration can easily be determined within a few minutes in colorimetric detection. This kit contains ready-to-use Coomassie Brilliant Blue G solution and BSA solution as a protein standard solution, and is suitable for a microplate assay. The protein detection range is from 10 µg/ml to 2,000 µg/ml by a standard method, and is from 1 µg/ml to 50 µg/ml by a micro method. Since the sensitivity of the CBB-based protein assay depends on the types of proteins be used, note the protein-to-protein variation in quantification.

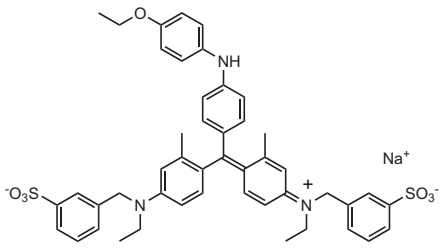

Fig.1 Chemical structure of Coomassie Brilliant Blue G

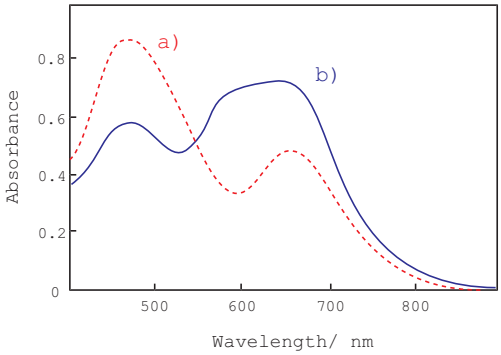

Fig. 2 Absorption spectra of CBB-G with and without protein.
a) protein free, b) protein (BSA): 500 µg/ml

Kit Contents

[500 tests]		[2500 tests]	
- CBB solution	100 ml x 2	- CBB solution	1 L x 1
- Standard BSA solution (4,000 µg/ml)	1.5 ml x 1	- Standard BSA solution (4,000 µg/ml)	1.5 ml x 2

Storage Condition

Store at 0-5°C
*CBB solution is stable for 12 months at 0-5°C and 6 months at room temperature.

Required Equipment and Materials

- Microplate reader (600 nm filter)
- 96-well microplate
- 10 µl, 100-200 µl pipettes
- Munti channel pipette
- 1.5 ml tube

Precaution

This kit contains glass vials. Handle them carefully.

General Protocol

Standard Method

1) Dilute Standard BSA solution with multiple dilution to prepare various concentration of Standard BSA solution.
   2,000 µg/ml, 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 63 µg/ml, 32 µg/ml, 0 µg/ml
2) Add 6 µl of various concentration of Standard BSA solution to each well.
3) Add 300 µl CBB solution to each well, and mix.
4) Vibrate the plate for 1 minute at room temperature.
5) Measure the absorbance of each well at 600 nm with a microplate reader.
6) Subtract the absorbance of the blank solution from the absorbance of each well.
7) Plot the concentration of BSA on the X-axis and absorbance on the Y-axis to prepare a calibration curve (Fig. 3).
   Determine protein concentration of unknown sample using the calibration curve.

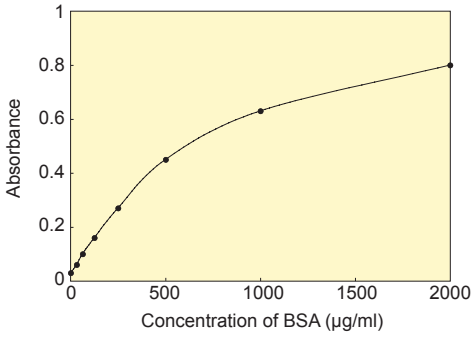

Fig. 3 Typical calibration curve prepared by Standard BSA solution.

Micro Method

Micro method is utilized for purified protein detection.[a)]

1) Dilute Standard BSA solution with multiple dilution to prepare various concentration of Standard BSA solution.
   50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.3 µg/ml, 3.2 µg/ml, 1.6 µg/ml, 0.8 µg/ml, 0 µg/ml
2) Add 150 µl CBB solution to each well.
3) Add 150 µl of various concentration of Standard BSA solution to each well, and mix.
4) Vibrate the plate for 1 minute at room temperature.

5) Measure the absorbance of each well at 600 nm with a microplate reader.
6) Subtract the absorbance of the blank solution from the absorbance of each well.
7) Plot the concentration of BSA on the X-axis and absorbance on the Y-axis to prepare a calibration curve. Determine protein concentration of unknown sample using the calibration curve.

a) Detergents severely interfere with micro assay.

Substances interfer with Standard Method

Certain substances interfere with the protein quantification assay including detergents, organic solvents chelating agents. Other substances may interfere with the assay if the concentration is too high. The maximum compatible concentrations of such substances are indicate in Table1.

Table 1 Compatible interfering material concentration[b)] in sample.

Chemical	Concentration
Detergent	
Brij 35	0.125 %
Brij 56	0.025 %
Brij 58	0.005 %
Triton X-100	0.125 %
Triton X-114	0.125 %
Tween 20	0.25 %
Tween 80	0.1 %
SDS	0.1 %
CHAPS	4 %
CHAPSO	4 %
MEGA 10	4 %
Octyl-β-D-glucoside	0.5 %
Organic sovent	
Ethanol	10 %
Isopropanol	10 %
DMSO	10 %
Chelating agent	
EDTA	0.4 mol/l
DTPA	0.4 mol/l

Chemical	Concentration
Salt	
Sodium chloride	2 mol/l
Potassium chloride	2 mol/l
Sodium acetate	0.4 mol/l
Sodium bicarbonate	0.1 mol/l
Buffer	
Citrate pH 5.0	0.125 mol/l
MES pH 6.1	0.125 mol/l
Tris pH 7.4	0.0625 mol/l
PBS	Undiluted
HEPES pH 7.5	0.125 mol/l
CHES pH 9.0	0.125 mol/l
Reducing agent	
Glucose	2 mol/l
Glutatione	0.04 mol/l
Ascorbic acid	0.4 mol/l
Dithiothreitol	0.01 mol/l

b) The compatible concentration was determined within ±5% fluctuation of the slope of the BSA calibration curve.

Protein-to-protein Variation

Standard BSA solution (4,000 µg/ml) is a standard protein to determine the protein concentration in samples. Protein-to-protein variation compared with BSA are shown in Table 2.

Table 2 Protein-to-protein variation in quantification

Protein	Protein vs. BSA [c)]
BSA	1.00
Chymotrypsinogen A	0.67
Transferrin	1.02
Human IgG	0.96

c) Value was determined by the comparison with the slope of calibration curve.

value=slope of protein/ slope of BSA

Notes

1. Since the sensitivity of the CBB-based protein assay depends on the type of proteins be used, note the protein-to-protein variation in quantification (Table 2). For more accurate quantification, use same protein as a standard.
2. Since CBB solution is highly acidic, handle with care.
3. If a protein concentration in a sample is too high, some proteins may precipitate from the solution mixture with CBB solution. Dilution of the sample solution is required prior to mix with CBB solution if protein concentration is high.
4. Confirm that excess amount of interfering materials is contained in the sample solution (see Table 1). If the amount of the interfering material is high, dilute it to reduce the concentration of interfering materials prior to use.

Reference

1) M. M. Bradford, *Anal. Biochem.*, **1976**, *72*, 248.

If you need more information, please contact Dojindo technical service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto 861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies,Inc.
Tel: +1-301-987-2667 Web:http://www.dojindo.com/
Dojindo EU GmbH
Tel: +49-89-3540-4805 Web: http://www.dojindo.eu.com/
Dojindo China Co., Ltd
Tel: +86-21-6427-2302 Web:http://www.dojindo.cn/