SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

# Protein quantification kit – general use

77371 (500 assays), 93736 (2500 assays)

### Application

For protein concentration determination, several methods, such as Lowry method, bicinchoninate method (BCA method), Biuret method and Bradford method, are available. Lowry and BCA methods are the most popular methods for protein quantification. Since both Lowry and BCA methods are based on the monovalent copper ion detection with monovalent copper ion specific chelating agents, a high concentration solution of divalent copper ion is required. Protein Quantification Kit-Wide Range is based on the reducing reaction of tetrazolium salt under basic conditions. Tetrazolium salt is easily reduced with sugar residues of proteins, and generates formazan dye. WST-8 formazan dye is yellow at neutral pH, and turns blue at higher pH. The maximum wavelength of the formazan dye is 650 nm at pH 12.5 or higher. The detection range of this kit is from 50 μg/ml to 5 mg/ml (BSA). Since the sensitivity of the protein assay depends on the type of proteins, please note the protein-to-protein variation in quantification.

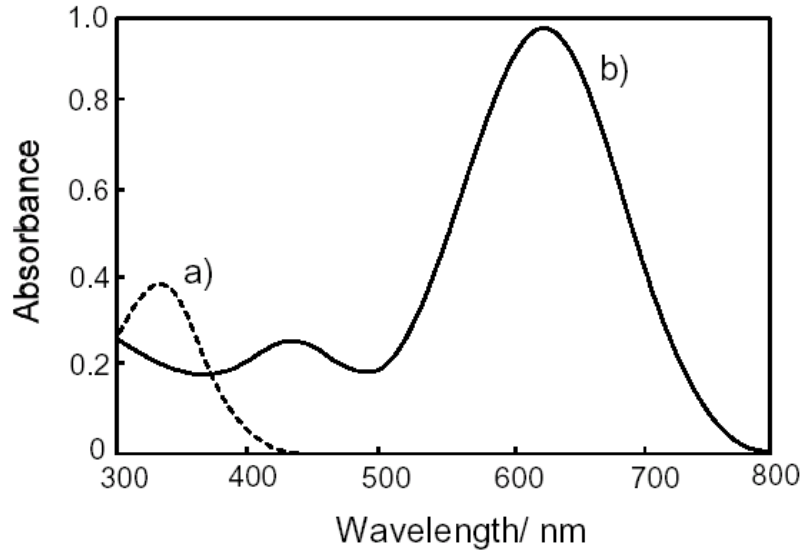


Figure 1. Absorption spectrum of WST-8
a) protein free
b) 2000 µg BSA/ml

## *Content*

| | |
|---|---|
| WST-8 Solution | 10 ml x 1 bottle |
| Buffer Solution | 100 ml x 1 bottle |
| BSA Standard Solution (10 mg/ml) | 1.5 ml x 1 vial |

**Storage**

Store the kit at 0-5 °C with protection from light. WST-8 Solution is stable for 12 months at 0-5 °C and 3 months at room temperature. Buffer Solution is stable for 18 months at room temperature. However, pH of Buffer Solution may drop due to carbon dioxide in the atmosphere. Please close the cap tightly after use. BSA Standard Solution is stable for 12 months at 0-5 °C.

**Equipment required**

1) Microplate reader (650 nm filter)
2) 96 well microplates
3) 10 l, 100-200 µl pipettes, multi-channel pipette

**Preparation of working solution**

Mix 100 µl of CCK-F with 5 ml of D-PBS (-) prior to use (1/50 dilution). Use 10 µl of the CCK-F/D-PBS (-) solution for 100 µl cell culture. 5 ml CCK-F working solution is sufficient for 5 plates (96-well). The CCK-F working solution should be used up in one day.

**Methods**

1) Dilute BSA Standard Solution using dd$H_2O$ with multiple dilutions to prepare various concentrations of BSA Standard solution (stock concentration of BSA Standard Solution is 10000 µg/ml). 5000 µg/ml, 2500 µg/ml, 1250 µg/ml, 625 µg/ml, 313 µg/ml, 156 µg/ml, 78 µg/ml, 0 µg/ml
2) Add 180 µl Buffer Solution to each well.
3) Add 20 µl of various concentrations of BSA Standard solution from Step 1 or sample solution to each well, and mix.
4) Add 20 µl WST-8 Solution to each well, and mix.
5) Cover the plate with an aluminum foil[a)] and incubate it at 37 °C for 30 min.
6) Measure the absorbance of each well at 630-670 nm with a microplate reader.
7) Subtract the absorbance of blank solution from the absorbance of each well.
8) Plot the concentration of BSA on the X-axis and the absorbance value on the Y-axis to prepare a calibration curve (a typical example is shown in Fig. 2). Determine the protein concentration of unknown sample using the calibration curve.

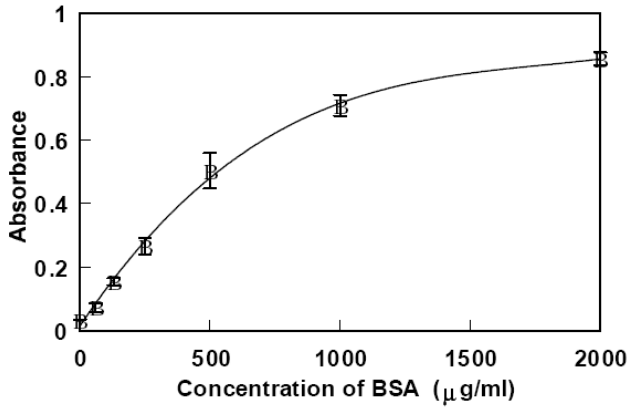


**Figure 2: Typical calibration curve**

Notes

1) Store the kit at 0-5 ◦C.
2) Since the sensitivity of CBB-based protein assays depends on the type of proteins, please note the protein-to-protein variation in quantification (Table 2). For more accurate quantification, use the same protein, which you want to measure, as the standard.
3) Since the Buffer Solution is highly basic, please handle with care.
4) The pH of the Buffer Solution may drop due to carbon dioxide in the atmosphere. Please close the cap tightly after use.
5) WST-8 is light sensitive after mixing with BufferSolution and the background of the solution may increase, so please protect from light during incubation.
6) Please 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.

**Materials That Interfere with the Assay**

The maximum non-interfering concentrations of materials, which may interfere with the protein quantification using Protein Quantification Kit – Wide Range, are shown in Table 1.

| **Category** | **Substance** | **Non-interfering concentration** |
|---|---|---|
| Detergent | Brij 35 | 2% |
| | Brij 56 | 1% |
| | Brij 58 | 1% |
| | Triton X-100 | 1% |
| | Triton X-114 | 1% |
| | Tween 20 | 0.5% |
| | Tween 80 | 0.3% |
| | SDS | 1% |
| | CHAPS | 4% |
| | CHAPSO | 2% |
| | MEGA 10 | 0.5% |
| | Octyl-®-D-glucoside | 0.5% |
| Organic solvent | Ethanol | 10% |
| | Isopropanol | 10% |
| | DMSO | 10% |
| Chelating agent | EDTA | 2.5 mM |
| | DTPA | 0.625 mM |
| Salt | Sodium chloride | 0.5 M |
| | Potassium chloride | 1 M |
| | Sodium acetate | 0.2 M |
| | Sodium bicarbonate | 6.25 mM |
| Buffer | Citrate pH 5.0 | 0.6 mM |
| | MES pH 6.1 | 12.5 mM |
| | Tris pH 7.4 | 2.5 mM |
| | PBS | no interference |
| | HEPES pH 7.5 | 12.5 mM |
| | CHES pH 9.0 | 12.5 mM |

Table 1: Compatible concentrationsa) of possible interfering materials with WST-8 assay

**Protein-to-protein Variation**

This kit determines the protein concentration of sample solution using BSA as a standard solution. Therefore, the concentration determined is not an absolute protein concentration. The protein-to-protein variation in quantification is shown in Table 2.

| Protein | Ratio protein/BSA |
|---|---|
| BSA | 1.00 |
| Chymotrypsinogen A | 0.75 |
| Transferrin | 0.97 |
| Human IgG | 0.37 |

Table 2: Protein-to-protein variation in quantification: Values were determined by the comparison with the slope of calibration curve*:* value=slope of protein/ slope of BSA

**Precautions and Disclaimer:**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.