Cat. # T9300A

For Research Use

TaKaRa

# TaKaRa BCA Protein Assay Kit

Product Manual

v201701Da

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## I. Description

The TaKaRa BCA Protein Assay Kit is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions. The Bicinchoninic Acid (BCA) Protein Assay is based on a two-step reaction in one tube. The first step consists of reduction of $Cu^{+2}$ to $Cu^{+}$ by peptide bonds of the protein in an alkaline medium (the biuret reaction). The second step is the formation of a purple-colored reaction product by the chelation of two molecules of BCA with one cuprous cation ($Cu^{+}$). This water soluble complex exhibits a strong absorbance at 562 nm and is nearly linear with increasing protein concentrations over a broad working range. The amount of protein present in a solution can be quantified by measuring absorption and comparing with protein solutions with known concentrations.

The BCA protein assay is not affected by the type of protein, exhibits linearity across a wide range of protein concentrations (0.02 - 2 mg/ml), and is resistant to the effects of surfactants. However, reducing agents and chelating agents may affect the reaction. For additional details, refer to Table 1 on Page 10.

Protein concentration is determined by constructing a standard curve based using the BSA Standard Solution that is included in the kit. This kit contains sufficient reagents for 500 assays for 1 ml reactions and 2,500 assays for 200 μl reactions in a microtiter plate.

## II. Components

| | | |
|---|---|---|
| 1. | BCA Reagent A | 250 ml x 2 |
| 2. | BCA Reagent B | 20 ml |
| 3. | BSA Standard Solution (2 mg/ml)* | 1 ml x 10 |

* The BSA Standard Solution contains 0.9% NaCl and 0.05% $NaN_3$ as stabilizers

## III. Storage

| | |
|---|---|
| BCA Reagent A, BCA Reagent B: | Room temperature |
| BSA Standard Solution: | 4℃ |

* This kit is shipped at room temperature. After receiving, please store the BSA Standard Solution at 4℃.

## IV. Materials Required but not Provided

- Spectrophotometer and compatible 1 ml cuvette
- Microplate reader and compatible microplate
- Benchtop centrifuge
- Microtubes (2 ml or 1.5 ml)
- Water bath (37℃)
- Incubator (60℃) (for the low-concentration measurement protocol)

## V. Precautions for Use

The following are precautions for use of this product. Please read before use.

- Before use, bring the BSA Standard Solution to room temperature or warm in a 20 - 50℃ water bath. After warming, vortex or tap lightly to mix well then briefly spin down.
- Deionized water, 0.9% NaCl, or PBS may be used for dilution of the measured samples and standards.
- BCA Reagent A and BCA Reagent B may precipitate at low temperatures. If this occurs, place at 20 - 37℃ , agitate lightly and use after the precipitate is completely dissolved.
- If a 562 nm filter is not available, perform measurement with a 540 - 570 nm filter. Doing so will have no effect on quantification.
- When measuring high-concentration samples, wash the cuvette well with water. Any pigment that builds up in the cuvette may affect subsequent measurement of low-concentration samples.

## VI. Protocol

There are five different protocols that may be used with this kit. Select the appropriate protocol based on the sample being measured.

VI-a. Standard protocol (protein range: 50 - 2000 μg/ml) (1 ml reaction)
VI-b. Standard protocol (protein range: 50 - 2000 μg/ml) (0.2 ml reaction)
VI-c. Low concentration protocol (protein range: 0 - 50 μg/ml) (1 ml reaction)
VI-d. Low concentration protocol (protein range: 0 - 50 μg/ml) (0.2 ml reaction/microtube)
VI-e. Low concentration protocol (protein range: 0 - 200 μg/ml) (0.2 ml reaction/microtiter plate)

**(Preparation of the Working Solution)**

Before measurement, prepare a working solution by mixing BCA Reagent A and BCA Reagent B at a 100:1 ratio. For example, to prepare 30 ml of working solution, add 0.3 ml of BCA Reagent B to 30 ml of BCA Reagent A and mix well.
The working solution is stable for 3 days at 4℃ after preparation.

＜ Required Volume of Working Solution ＞

The amount of working solution needed can be calculated as follows:

Total amount (ml) of working solution necessary = [(7 or 8 BSA standard curve samples) x Number of replicates (n) + 1] x amount of working solution per reaction

Example (protocol VI-a.): Measurement will be performed in duplicate (n=2) for 12 samples using the standard protocol (1 ml reaction):
[(8 + 12) x 2 + 1 ] x 1 ml = 41 ml

Example (protocol VI-b.): Measurement will be performed in duplicate (n=2) for 20 samples using the standard protocol (0.2 ml reaction):
[(8 + 20) x 2 + 1 ] x 0.2 ml = 11.4 ml

Example (VI-c.): Measurement will be performed for in duplicate (n=2) for 12 samples using the low concentration protocol (1 ml reaction):
[(7 + 12) x 2+ 1 ] x 0.5 ml = 19.5 ml

### VI-a. Standard Protocol (range: 50 - 2000 μg/ml; 1 ml reaction)

1) Prepare dilutions of the BSA standard solution as shown below. Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the sample.

| 2 mg/ml BSA standard (μl) | Diluent (μl) | Final concentration of BSA (μg/ml) |
|---|---|---|
| 120 | 0 | 2,000 |
| 90 | 30 | 1,500 |
| 60 | 60 | 1,000 |
| 45 | 75 | 750 |
| 30 | 90 | 500 |
| 15 | 105 | 250 |
| 10 | 150 | 125 |
| 0 | 120 | 0 (Blank) |

2) Standard Curve
   1. Dispense 50 μl of each dilution of the BSA standard solution into a microtubes. Perform at least 2 replicate measurements (n=2) for each concentration.
   2. Add 1 ml of the working solution and mix immediately.
   3. Incubate for 30 minutes in a 37℃ water bath and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 1 ml cuvettes for measurement. Zero the instrument with a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 20 min.
   5. Subtract the average value of blank replicates from the absorbance for all other individual standard measurement, and generate the standard curve.

3) Sample Measurement
   It is recommend that samples are measured at the same time as the BSA standards.

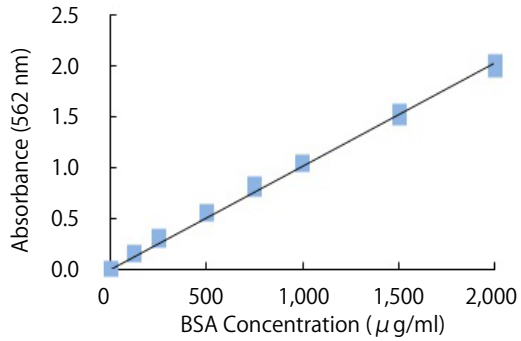


Figure 1. Standard Curve for BSA from 0 - 2,000 μg/ml

   1. Dispense 50 μl of each sample into 1.5 ml microtubes. Perform at least duplicate measurements for each sample. It is also possible to prepare a serial dilution of a sample in the same manner as the standard solution for measurement.
   2. Add 1 ml of the working solution and mix immediately.
   3. Incubate for 30 minutes in a 37℃ water bath and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 1 ml cuvettes for measurement. Zero with water. Subsequently, measure the absorbance of all the samples within 20 min.
   5. Subtract the value of the blank from the absorbance and determine the concentration of the sample using the standard curve as a reference.

**VI-b. Standard Protocol (range: 50 - 2000 μg/ml; 0.2 ml volume)**

1) Prepare dilutions of BSA standard solution as in VI-a-1). Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the samples.
2) Standard Curve
   1. Dispense each dilution of the BSA standard solution into microtubes or the wells of a microtiter plate. Use 10 μl each when using microcuvettes or 25 μl when using a microtiter plate. Perform at least duplicate measurements (n=2) for each concentration.
   2. Add 200 μl of the working solution and mix immediately.
   3. Incubate for 30 minutes in a 37℃ water bath and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 0.2 ml microcuvettes with an optical path length of 1 cm for measurement. For a microtiter plate, use a plate reader to measure absorbance at 562 nm. Zero with water. Subsequently, measure the absorbance of all the samples within 20 min.
   5. Subtract the average value of blank replicates from the absorbance for all other individual standard measurement, and generate the standard curve.
3) Sample Measurement
   It is recommend that samples are measured at the same time as the BSA standards.
   1. Dispense the samples into microtubes or the wells of a microtiter plate. Use10 μl when using microcuvettes or 25 μl when using a microtiter plate. Perform at least duplicate measurements (n=2) for each sample. It is also possible to prepare a serial dilution of a sample in the same manner as the standard solution for measurement.
   2. Add 200 μl of the working solution and mix immediately.
   3. Incubate for 30 minutes in a 37℃ water bath and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 0.2 ml microcuvettes with an optical path length of 1 cm for measurement. For a microtiter plate, use a plate reader to measure absorbance at 562 nm. Use water as a zero blank.
   5. Subtract the value of the blank from the absorbance and determine the concentration of the sample using the standard curve as a reference.

### VI-c. Low-Concentration Protocol (range: 0 - 50 μg/ml; 1 ml reaction)

1) Preparation of the BSA Standard Solution
   1. Mix 100 μl of the BSA Standard Solution (2 mg/ml) with 900 μl of diluent and mix well to prepare the 0.2 mg/ml BSA standard solution.
   2. Prepare 2 sets of the BSA standard dilution series shown below in 1.5 ml microtubes (7 types x 2 sets =14 microtubes). Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the samples.

| 0.2 mg/ml BSA standard (μl) | Diluent (μl) | Final concentration of BSA (μg/ml) | Final volume (μl) |
|---|---|---|---|
| 125 | 375 | 50 | 500 |
| 100 | 400 | 40 | 500 |
| 75 | 425 | 30 | 500 |
| 50 | 450 | 20 | 500 |
| 25 | 475 | 10 | 500 |
| 12.5 | 487.5 | 5 | 500 |
| 0 | 500 | 0 (Blank) | 500 |

2) Standard Curve
   1. Directly add 500 μl of working solution to the dilutions of BSA and mix immediately.
   2. Incubate for 60 minutes at 60℃ and then return to room temperature.
   3. Measure absorbance at 562 nm using a spectrophotometer. Use 1 ml cuvettes for measurement. Zero with water. Subsequently, measure the absorbance of all the samples within 20 min.
   4. Subtract the average value of blank replicates from the absorbance for all other individual standard measurement, and generate the standard curve.
3) Sample Measurement
   It is recommend that samples are measured at the same time as the BSA standards.
   1. Dispense 500 μl of each sample into 1.5 ml microtubes. Perform at least duplicate measurements (n=2) for each sample. It is also possible to prepare a serial dilution of a sample in the same manner as the standard solution for measurement.
   2. Add 500 μl of the working solution and mix immediately.
   3. Incubate for 60 minutes at 60℃ and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 1 ml cuvettes for measurement. Zero with water. Subsequently, measure the absorbance of all the samples within 20 min.
   5. Subtract the value of the blank from the absorbance and determine the concentration of the sample using the standard curve as a reference.

### VI-d. Low-Concentration Protocol (range: 0 - 50 μg/ml; 0.2 ml reaction/microtube)

1) Preparation of the BSA Standard Solution
   1. Mix 100 μl of the BSA Standard Solution (2 mg/ml) with 900 μl of diluent and mix well to prepare the 0.2 mg/ml BSA standard solution.
   2. Prepare 2 sets of the BSA standard solution dilution series shown below in 1.5 ml microtubes (7 types x 2 sets = 14 microtubes). Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the samples.

| 0.2 mg/ml BSA standard (μl) | Diluent (μl) | Final concentration of BSA (μg/ml) |
|---|---|---|
| 125 | 375 | 50 |
| 100 | 400 | 40 |
| 75 | 425 | 30 |
| 50 | 450 | 20 |
| 25 | 475 | 10 |
| 12.5 | 487.5 | 5 |
| 0 | 500 | 0 (Blank) |

2) Standard Curve
   1. Dispense 100 μl each of the dilutions into microtubes. Perform at least duplicate measurements (n=2) for each concentration.
   2. Add 100 μl of the working solution and mix immediately.
   3. Incubate for 60 minutes at 60℃ and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 0.2 ml microcuvettes for measurement, with an optical path length of 1 cm. Use water as a zero blank.
   5. Subtract the average value of blank replicates from the absorbance for all other individual standard measurements, and generate the standard curve.
3) Sample Measurement
   It is recommend that samples are measured at the same time as the BSA standards.
   1. Dispense 100 μl of each sample into microtubes. Perform at least duplicate measurements (n=2) for each sample. It is also possible to prepare a serial dilution of a sample in the same manner as the standard solution for measurement.
   2. Add 100 μl of the working solution and mix immediately.
   3. Incubate for 60 minutes at 60℃ and then return to room temperature.
   4. Measure absorbance at 562 nm using a spectrophotometer. Use 0.2 ml microcuvettes with an optical path length of 1 cm for measurement. Zero with water.
   5. Subtract the value of the blank from the absorbance and determine the concentration of the sample using the standard curve as a reference.

### VI-e. Low-Concentration Protocol (range: 0 - 200 μg/ml; 0.2 ml reaction/microtiter plate)

1) Preparation of the BSA Standard Solution
   1. Mix 120 μl of the BSA Standard Solution (2 mg/ml) with 1,080 μl of diluent and mix well to prepare the 0.2 mg/ml BSA standard solution.
   2. Prepare dilutions of the BSA standard solution as shown below. Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the samples.

| 0.2 mg/ml BSA standard (μl) | Diluent (μl) | Final concentration of BSA (μg/ml) |
|---|---|---|
| 400 | 0 | 200 |
| 300 | 100 | 150 |
| 200 | 200 | 100 |
| 100 | 300 | 50 |
| 40 | 360 | 20 |
| 20 | 380 | 10 |
| 10 | 390 | 5 |
| 0 | 400 | 0 (Blank) |

2) Standard Curve
   1. Dispense 100 μl each of the dilutions of BSA standard solution into the wells of the microtiter plate. Perform at least duplicate measurements (n=2) for each concentration.
   2. Add 100 μl of the working solution and mix immediately.
   3. Incubate for 60 minutes at 37℃ and then return to room temperature after the reaction.
   4. Measure absorbance at 562 nm using a plate reader. Use water as a zero blank.
   5. Subtract the average value of blank replicates from the absorbance for all other individual standard measurements, and generate the standard curve.

3) Sample Measurement
   It is recommend that samples are measured at the same time as the BSA standards.
   1. Dispense 100 μl of each sample into microtubes. Perform at least duplicate measurements (n=2) for each sample. It is also possible to prepare a serial dilution of a sample in the same manner as the standard solution for measurement.
   2. Add 100 μl of the working solution and mix immediately.
   3. Incubate for 60 minutes at 37℃ and then return to room temperature.
   4. Measure absorbance at 562 nm using a plate reader. Use water as a blank.
   5. Subtract the value of the blank from the absorbance and determine the concentration of the sample using the standard curve as a reference.

## VII. Appendix

### Effects of Coexisting Substances

The BCA method is comparatively resistant to the effects of substances such as surfactants and buffers; however, high concentrations of various components affect measurement. The concentrations at which measurement with this kit is unaffected are shown in Table 1.

Table 1. Permissible Concentrations of Various Reagents under the Standard Protocol

| Substance | Permissible Concentration of the Coexisting Substance |
|---|---|
| Salts / Buffers | |
| Ammonium sulfate | 0.15 M |
| Borate pH 9.5 | 50 mM |
| Calcium chloride | 10 mM |
| Glycine | 100 mM |
| Guanidine-HCl | 4 M |
| HEPES, pH 7.5 | 100 mM |
| Imidazole, pH 7.0 | 50 mM |
| KPB, pH 7.0 | 100 mM |
| Magnesium chloride | 10 mM |
| MES, pH 6.1 | 100 mM |
| MOPS, pH 7.2 | 100 mM |
| NaPB, pH 7.0 | 100 mM |
| Nickel chloride | 10 mM |
| PIPES, pH 6.8 | 100 mM |
| Sodium acetate, pH 5.0 | 200 mM |
| Sodium azide | 0.20% |
| Sodium chloride | 1 M |
| Sodium citrate, pH 6.4 | 100 mM |
| Tricine, pH 8.0 | 25 mM |
| Tris-HCl, pH 8.0 | 50 mM |
| Zinc chloride | 10 mM |
| Detergents | |
| Brij-35 | 5% |
| CHAPS | 5% |
| Nonidet P-40 | 5% |
| Triton X-100 | 5% |
| Tween-20 | 5% |
| SDS | 5% |

| Substance | Permissible Concentration of the Coexisting Substance |
|---|---|
| Chelating agents | |
| EDTA | 10 mM |
| EGTA | 1 mM |
| Reducing agents | |
| Cysteine | 1 mM |
| Dithiothreitol | 1 mM |
| Glucose | 10 mM |
| 2-Mercaptoethanol | 0.01% |
| Organic solvents | |
| Acetone | 10% |
| DMSO | 10% |
| Ethanol | 10% |
| Methanol | 10% |
| Misc. Reagents | |
| Glycerol | 50% |
| Hydrochloric Acid | 100 mM |
| PMSF | 1 mM |
| Sodium Hydroxide | 100 mM |
| Urea | 3 M |

**Effects of Protein Type**

The BCA method is not significantly affected by protein type. Figure 2 shows a standard curve prepared by measuring serial dilutions of the conventionally-used standard substances BSA and BGG. Table 2 shows the ratio of coloration of 15 typical proteins when compared with BSA.

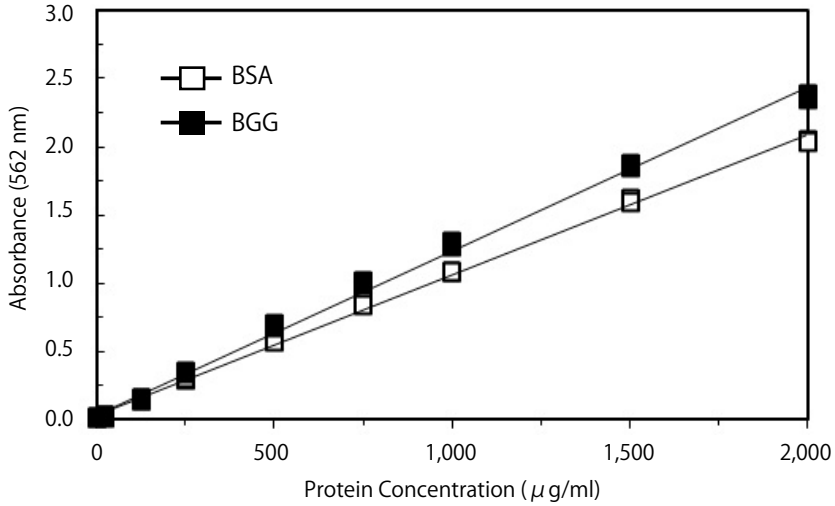


Figure 2. Standard Curves for BSA and BGG

Table 2. Coloration Ratios of Various Proteins to BSA Using the Standard Protocol

| Protein | Ratio* |
|---|---|
| Albumin, bovine serum (BSA) | 1.00 |
| Alcohol Dehydrogenase, *Saccharomyces cerevisiae* | 0.54 |
| Aldolase, rabbit muscle | 0.89 |
| Carbonic Anhydrase, bovine erythrocytes | 0.77 |
| a-Chymotrypsin, bovine pancreas | 1.06 |
| Cytochrome C, bovine heart | 0.90 |
| Gamma globulin, bovine (BGG) | 1.16 |
| Hemoglobin, bovine | 0.66 |
| IgG, rabbit | 1.29 |
| IgG, mouse | 1.15 |
| Insulin, human | 1.28 |
| Lysozyme, chicken egg white | 1.19 |
| Ovalbumin, chicken egg white | 0.95 |
| Transferrin | 0.85 |
| Trypsin | 0.98 |

* Ratio = (Mean net absorbance of individual proteins) / (Mean net Absorbance of BSA)

## VIII. Related Products

TaKaRa Bradford Protein Assay Kit (Cat. #T9310A)