BCA Quantification Kit (Broad Range)

#GQ05.0500

(FOR RESEARCH ONLY)

Product: The Bicinchoninic Acid (BCA) protein assay for the quantification of proteins is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions. Similar to the Lowry method, the principle is based on the Biuret reaction in which Cu^{2+}-protein complexes are formed under alkaline condition. As proteins react as reducing agents, Cu^{2+} is reduced to Cu^{+}, which is subsequently chelated by two BCA molecules, forming a purple complex that exhibits strong absorption at 562 nm. As the amount of Cu^{+} is proportional to the amount of protein, measuring absorption can be used to quantify protein concentration by comparing with the absorption of protein solutions of known concentrations. In comparison with the Lowry method, the BCA method is not only easier and faster, but also suffers less interference from non-ionic detergents and salts. It is insensitive to the presence of detergents such as SDS (1%) and Triton X-100.

GRiSP´s BCA Quantification Kit (Broad Range) is formulated for the quantification of total protein concentration ranging from 20-2000µg/ml. It is suitable for usage with standard spectrophotometers as well as with microplate readers. The whole procedure can be carried out in less than an hour.

Applications: Protein Quantification (Broad Range: 20-2000µg/ml)

Contents: One kit is sufficient for 2500 assays using microplates and reader or 250 assays using test tubes and a standard spectrophotometer.

Component	GQ05.0500
Solution A (1% BCA/tartrate in alkaline carbonate buffer)	2x 250 ml
Solution B (4% $CuSO_4.5H_2O$ in water (w/w))	15 ml
Protein Standard [BSA (2000 µg/ml)]*)	1 ml

*) see page 2, section "Protein Standard for Calibration"

Storage: Store Solutions A and B at room temperature, and Protein Standard at -20°C for at least 18 months. Precipitation may occur in Solutions A and B. Simply redissolve by gentle warming and stirring.

Protein Standard:

The BCA method is based on the comparison of the absorption of an unknown sample to the absorptions of a serial dilution of a standard protein, most commonly Bovine Serum Albumin (BSA) with known concentration, which are used to prepare a calibration curve. Proteins are diverse in many ways, including in amino acid sequence, secondary structure, isoelectric point, prosthetic groups and so on. One major advantage of the BCA method, in comparison with other methods, is that it exhibits less protein-to-protein variation, as it is based on the amount of peptide bonds. However, variation in disulfide bonds and reducing amino acids (Cys, Tyr, Trp) does have some influence on the reduction of Cu^{2+} to Cu^{+} and thus on absorption. Therefore, some proteins absorb more light (e.g. Lysozyme and IgG), whereas other proteins (e.g. Collagen and Ovalbumin) absorb less. This means that if one uses BSA for the preparation of a calibration curve for the measurement of IgG, there is an overestimation of the amount of IgG present and in case of Ovalbumin there would be an underestimation. Ideally, one would use the same protein as under investigation for the preparation of a calibration curve. Since this is often not possible, BSA is used as standard. Hereunder one can find a table with reported correction factors for some proteins, in comparison with BSA (factor 1.00).

Protein	Factor		Protein	Factor
Human IgG	1.09		Collagen (Type I/II)	0.4-0.6
Rabbit IgG	1.12		Cytochrome C (horse)	0.74
Sheep IgG	1.17		Aldolase (rabbit)	0.85
Mouse IgG	1.18		Transferrin (human)	0.89
Lysozyme	1.37		Ovalbumin	0.93

For convenience, GRiSP´s BCA Quantification Kit (Broad Range) is supplied with one vial containing 1ml of 2000 µg/ml of BSA. If required, it is possible to purchase BSA standard separately.

Prior to Use:

a) Preparation of standards

In order to prepare a calibration curve, it is recommended to prepare a fresh set of protein standards from a 2000µg/ml stock solution, ranging from 20-2000µg/ml range, ideally prepared in the same buffer as found in the test samples. It is recommended to prepare sufficient to measure each standard in duplicate or triplicate. It is necessary to make a calibration curve every time you run a new assay, regardless of the format (spectrophotometer or microplate reader). One suggestion to prepare the standards (for triplicates, using protocol for spectrophotometer) is as follows:

1) label 8 test tubes (A – H) and add 300µl of stock protein solution (2000µg/ml) to tube A ($[BSA]_f$ = 2000µg/ml)
2) add 375µl of stock protein solution (2000µg/ml) to tube B and dilute/mix with 125µl of buffer ($[BSA]_f$ = 1500µg/ml)
3) add 325µl of stock protein solution (2000µg/ml) to tube C and dilute/mix with 125µl of buffer ($[BSA]_f$ = 1000µg/ml)
4) add 175µl of tube B to tube D and dilute/mix with 175µl of buffer ($[BSA]_f$ = 750µg/ml)
5) add 325µl of tube C to tube E and dilute/mix with 325µl of buffer ($[BSA]_f$ = 500µg/ml)
6) add 325µl of tube E to tube F and dilute/mix with 325µl of buffer ($[BSA]_f$ = 250µg/ml)
7) add 325µl of tube F to tube G and dilute/mix with 325µl of buffer ($[BSA]_f$ = 125µg/ml)
8) add 100µl of tube G to tube H and dilute/mix with 400µl of buffer ($[BSA]_f$ = 25µg/ml)

This requires in total 1ml of a 2000µg/ml stock solution. If one were to use a microplate reader, 250µl would be sufficient (for triplicate).

b) Preparation of BCA Working Solution

Determine the amount of BCA Working Solution required. In case of using a spectrophotometer, this is 2.0 ml per sample/standard and in case of using a microplate reader this is 200µl per sample/standard. In case of N samples, prepare for N+5, as one needs to include a "blank" in triplicate and account for pipetting errors. In order to prepare BCA Working Solution, add 1 volume of Solution B to 50 volumes of Solution A and mix well.

Protocol for Spectrophotometer

1. Pipet 100µl of each standard and unknown sample (in replicates) into an appropriate labeled tube. Also include a "blank", containing only the buffer used for the preparation of samples and standards.
2. Add 2.0ml of the BCA Working Solution (see prior to use) and mix well.
3. Cover the tubes and incubate in a water bath at 37ºC for 30 minutes (or alternatively at room temperature for 2 hours).
4. Allow to cool to room temperature
5. Set the spectrophotometer, according to the manufacturer´s instructions to read at 562nm.
6. Zero the instrument using a cuvette filled with ddH_2O (this is not the "blank"!)
7. Read the absorbance of the blank, all the standards, and samples in the shortest possible time, preferably within 15 minutes. (The color development continues even at room temperature, which may affect accuracy if measurements are delayed).

Protocol for Microplate Reader

1. Pipet 25µl of each standard and unknown sample (in replicates) into an appropriate labeled tube. Also include a "blank", containing only the buffer used for the preparation of samples and standards.
2. Add 200µl of the BCA Working Solution (see prior to use) and mix well using a plate shaker for 30 sec.
3. Cover the plate and incubate at 37ºC for 30 minutes
4. Allow to cool to room temperature
5. Read the absorbance at 562nm (or near 562nm) according to the manufacturer´s instructions.

Quantification

1. Determine the average value of the "blank" standard replicates and subtract this value from all other measurements.
2. Plot the average "blank-corrected" values for each protein standard against its known concentration (in µg/ml), and construct a standard calibration curve.
3. Use the standard curve to determine the protein concentration of each unknown sample and calculate the original sample concentration taking into account all additional dilution steps, if carried out.
4. In case the correction factor for the protein in the unknown sample is known (see page 2), one should divide the final outcome by the correction factor in order to prevent over or underestimation.

Compatibility Troubleshooting

GRiSP´s BCA Quantification Kit (Broad Range) is compatible with the following detergents up to the given concentrations: 5% Brij®-35, 1% Brij®-56, 1% Brij®-58, 5% CHAPS, 5% CHAPSO, 5% Deoxycholic acid, 5% Nonidet P-40 (NP-40), 5% Octyl ß-glucoside, 5% Octyl ß-thioglucopyranoside, 5% SDS, 1% Span® 20, 5% Triton® X-100, 1% Triton® X-114, 1% Triton® X-305, 1% Triton® X-405, 5% Tween®-20, 5% Tween®-60, 5% Tween®-80 and 1% Zwittergent® 3-14.
It is also not interfered by the following reducing and thiol containing agents up to the given concentrations: 10% N-acetylglucosamine (in PBS), 1mM DTE, 1mM DTT, 10mM Glucose, 0.01 ß-mercaptoethanol, 3M Potassium thiocyanate and 0.01% Thimerosal. Moreover, the presence of chelating agents EDTA up to 10mM and Sodium Citrate (up to 200mM) have no negative effect on the outcome of the assay.

In general, chelating reagents or reagents that change the pH or reduce copper are known to interfere with the assay. These include ascorbic acid, cathecholamines, creatinine, cysteine, EGTA, glycerol (impure), hydrogen peroxide, hydrazides, iron, lipids, melibiose, phenol red, sucrose (impure), tryptophan, tyrosine and uric acid.

Troubleshooting

1. **No Color Development**

   A possible cause is the presence of chelating agents present in the sample.
   A possible solution is to dialyze and/or desalt the sample.
   Another option is to increase the amount of copper in the BCA Working Solution by preparing a mix of 50:2 or 50:3 instead of 50:1 (A:B). This needs to be applied then to all samples and standards.

2. **Weaker Color Development than expected**

   This can be caused by strongly altered pH, either by strong acid or strong alkaline buffer
   A possible solution is to dialyze and/or desalt and/or dilute the sample

3. **Stronger Color Development than anticipated**

   A possible cause is a too high protein concentration, in which case on can dilute the sample
   Another possible reason could be the presence of lipids or lipoproteins. The interference can be eliminated by adding 2% SDS to the sample

4. **All tubes are dark purple**

   Reducing agents or Thiols are present in the sample buffer
   A possible solution is to dialyze and/or dilute the sample