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Product Information

QuantiPro™ BCA Assay Kit

Catalog Number **QPBCA**

TECHNICAL BULLETIN

Product Description

Protein determination is one of the most common operations performed in biochemical research. The principle of the bicinchoninic acid (BCA) assay is similar to the Lowry procedure,[1] in that both rely on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to protein present. It has been shown that cysteine, cystine, tryptophan, tyrosine, and the peptide bond[2] are able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.[3]

The BCA assay is more sensitive and applicable than either biuret or Lowry procedures. In addition, it has less variability than the Bradford assay. The BCA assay has many advantages over other protein determination techniques:

- It is easy to use.
- The color complex is stable.
- There is less susceptibility to detergents.
- It is applicable over a broad range of protein concentrations.

The QuantiPro™ BCA Assay Kit is based on the same principals as the BCA1 Kit, except the QuantiPro BCA Kit gives a linear response from 0.5 to 30 μg/ml of protein, while the BCA1 Kit gives a linear response at higher concentrations of protein (200-1,000 μg/ml). Since the QuantiPro BCA assay can detect low concentrations of protein in small volumes of solution, it is recommended for use in multiwell plate assays.

In addition to protein determination in solution, the QuantiPro BCA protein assay has other applications, including determination of protein covalently bound to agarose supports and protein adsorbed to multiwell plates. More recently this procedure has been applied to the determination of functional groups such as sulfhydryl, N-hydroxysuccinimido carboxylate, aldehyde, and hydrazide on a variety of solid supports.

Components

QuantiPro Buffer QA (M3810)
Reagent QA is supplied in a square plastic bottle containing 250 ml of a solution consisting of sodium carbonate, sodium tartate, and sodium bicarbonate in 0.2 M NaOH, pH 11.25.

QuantiPro BCA QB (M3685)
Reagent QB is supplied in a square plastic bottle containing 250 ml of a 4% (w/v) bicinchoninic acid solution, pH 8.5.

Copper(II) sulfate, Pentahydrate 4% Solution (C2284)
Reagent QC is supplied in a plastic bottle containing 12 ml of a 4% (w/v) copper(II) sulfate, pentahydrate solution.

Protein Standard Solution (P0914)
This product is supplied in 10 flame-sealed glass ampules, each containing 1.0 ml of a solution consisting of 1.0 mg/ml bovine serum albumin in 0.15 M NaCl with 0.05% sodium azide as a preservative.

Reagents and Equipment Required But Not Provided

- Spectrophotometer capable of measuring absorbance in the 560 nm region.
- Test tubes, 13 × 100 mm
- 1.5 ml Capped Tubes, Catalog Number T9661
- 96 well plates
- 384 well plates
- Disposable Plastic Cuvets, Catalog Number C5416

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.

Preparation Instructions
The QuantiPro Working Reagent is prepared by mixing 25 parts of Reagent QA with 25 parts of Reagent QB. After Reagents QA and QB have been combined, add 1 part of Reagent QC (copper(II) sulfate) and mix until it is uniform in color.

Storage/Stability
Store Reagents QA, QB, and QC at room temperature. Reagents QA and QB can be mixed together without Reagent QC, and stored in a closed container at room temperature for a week. If this kit is received or stored cold, a precipitate may form in Reagent QA or Reagent QB. To dissolve the precipitate, warm the solution slowly to room temperature while mixing. The solutions are now suitable for use.

Store the Protein Standard Solution at 2–8 °C.

Procedure
The QuantiPro assay consists of mixing 1 part of a protein sample with 1 part of the prepared QuantiPro Working Reagent. The protein sample is either a blank, a protein standard, or an unknown sample. The blank consists of sample buffer with no protein. The protein standard consists of a known concentration of protein, and the unknown sample is the solution to be quantified.

QuantiPro assays are routinely performed at 60 °C. Color development begins immediately, but can be slowed down by incubation at lower temperatures (see Procedures A and B). The absorbance at 562 nm is recorded and the protein concentration is determined by comparison to a standard curve.

A. Standard 2 ml Assay Protocol
This is the standard assay that can be performed in a test tube. This procedure uses 1 ml of a protein sample and 1 ml of the mixed QuantiPro Working Reagent. These instructions are a step by step procedure on how to perform the standard assay. If a nonstandard assay is used (i.e., 96 well plate) adjust the volumes accordingly.

1. Prepare the required amount of QuantiPro Working Reagent needed for the assays (see Table 1). The final volume used in the assay depends upon the application and the equipment available. Table 1 can be used to determine the volume of QuantiPro Working Reagent to prepare, depending on how many blanks, protein standards, and unknown samples are to be assayed.
2. Combine the volumes of Reagents QA and QB specified in the table and then add the stated amount of Reagent QC (copper(II) sulfate). Mix until the QuantiPro Working Reagent is a uniform, light green color.

Table 1.
Volume of QuantiPro Working Reagent to prepare. This is dependent on how many blanks, protein standards, and unknown samples are to be assayed.

Number of Assays				Amount of Each Reagent Used			
Number of 2 ml Standard Test tube assays	Number of 1 ml micro-centrifuge tube assays	Number of 300 µl assays in a 96 well plate	Number of 80 µl assays in a 384 well plate	QA (ml)	QB (ml)	Copper(II) Sulfate Reagent QC (ml)	Total amount of QuantiPro Working Reagent (ml)
1	2	6	25	0.5	0.5	0.02	1.02
5	10	34	127	2.5	2.5	0.1	5.1
10	20	68	255	5.0	5.0	0.2	10.2
14	29	**96**	369	7.25	7.25	0.29	14.79
15	31	105	**384**	7.75	7.75	0.31	15.81

2. Prepare 1 ml protein standards of appropriate concentrations in the same buffer as the unknown sample. Deionized water maybe used as a substitute for the buffer, but any interference due to the buffer will not be compensated for in the protein standards. These standards can range from 0.5 to 30 µg/ml. Create a standard assay setup table similar to Tables 2a or 2b. Each tube should contain 1 ml of a known standard, blank (buffer only), or unknown sample.

<u>EXAMPLES of Standard Assay Set-Up Tables</u>
Table 2a.
If equipment is not available to handle volumes of liquid less than 10 µl accurately, it will be necessary to prepare a second protein standard solution (50 µg/ml). To do this, first pipette 1.9 ml of buffer into a test tube and then add 100 µl of the 1 mg/ml protein standard (P0914) into the test tube. The concentration of this 2 ml solution is 50 µg/ml. Prepare protein standards as described (Tubes 1-6) using the newly created 50 µg/ml protein standard. The new 50 µg/ml standard can be stored in a plastic container at 4 °C for a week depending on the storage buffer used. Make a fresh 50 µg/ml protein standard if this standard becomes contaminated.

For protein samples with unknown concentrations, it may be necessary prepare a dilution scheme to ensure the concentration is within the linear range of 0.5–30 µg/ml. An unknown sample scheme is represented in Table 2a by tubes 7-9. Tube 7 is the unknown sample without dilution, while tubes 8 and 9 are 2-fold and 10-fold dilutions, respectively (see table). Researchers must determine their own scheme based on their estimation of the concentration of the unknown sample.

Tube No.	Sample Buffer (µl)	Protein Standard Solution (50 µg/ml) (µl)	Unknown sample (µl)	[Protein] (µg/ml)	BCA Working Reagent (µl)
1	1,000	–	–	0	1,000
2	990	10	–	0.5	1,000
3	900	100	–	5	1,000
4	800	200	–	10	1,000
5	600	400	–	20	1,000
6	400	600	–	30	1,000
7	0	–	1,000	–	1,000
8	500	–	500	–	1,000
9	900	–	100	–	1,000

Table 2b.
If a Hamilton syringe or other equipment suitable for handling volumes of less than 10 µl accurately is available, the protein standards can be setup as described using the original 1 mg/ml standard (P0914).

A scheme for the unknown samples may be setup in a manner similar to that described in Table 2a.

Tube No.	Sample Buffer (µl)	Protein Standard (1 mg/ml) (µl)	Unknown sample (µl)	[Protein] (µg/ml)	BCA Working Reagent (µl)
1	1,000	–	–	0	1,000
2	999.5	0.5	–	0.5	1,000
3	995	5	–	5	1,000
4	990	10	–	10	1,000
5	980	20	–	20	1,000
6	970	30	–	30	1,000
7	0	–	1,000	–	1,000
8	500	–	500	–	1,000
9	900	–	100	–	1,000

3. Add 1 ml of the QuantiPro Working Reagent to 1 ml of each protein standard, blank, and unknown sample. Vortex gently for thorough mixing. The total liquid volume in the test tube is 2 ml.

4. The following incubation parameters may be used:
   60 °C for 1 hour **Or**
   37 °C for 2 hours **Or**
   25 °C (Room Temperature) for 16 hours (overnight).

5. Allow the tubes to cool to room temperature and measure the absorbance of the reaction solution at 562 nm. Color development continues slowly after cooling to room temperature, but no significant error is seen if all the tubes are read within 10 minutes of each other. Create an assay table as needed and a standard curve (Examples are shown in the results below).

6. Determine protein concentration by comparison of the absorbance of the unknown samples to the standard curve prepared using the protein standards.

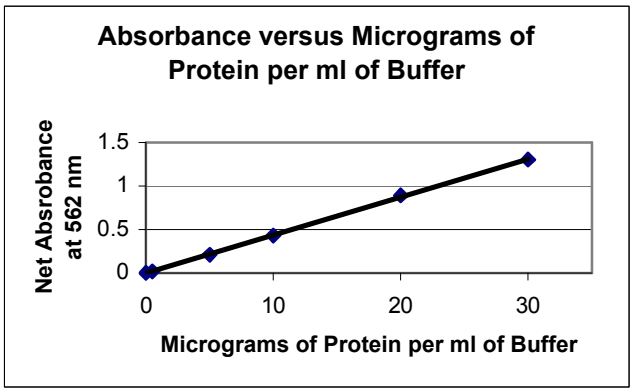
Results Based on the Standard Assay
Create a table with the absorbance results obtained during the assay. The results you get for the absorbance of the protein standards may differ from those presented here.

Table 3. EXAMPLE - Assay Data Table

Tube No.	A_{562}	Net A_{562}	[Protein Sample Assayed] (µg/ml)	Dilution Factor	[Protein Unknown sample] (µg/ml)
1	0.1	0	0	1	-
2	0.14	0.04	0.5	1	-
3	0.31	0.21	5	1	-
4	0.53	0.43	10	1	-
5	0.99	0.89	20	1	-
6	1.51	1.41	30	1	-
7	0.98	0.88	20	1	20
8	0.49	0.39	10	2	20
9	0.19	0.09	2	10	20

The Net Absorbance at 562 nm (Net A_{562}) is found by subtracting the absorbance (A_{562}) of the blank (Tube 1) from the recorded A_{562} values for the protein standards and unknown samples. Create a standard curve (see Graph 1 on the next page) by plotting the Net Absorbance at 562 nm versus the protein standard concentrations (µg/ml, Tubes 1-6).

Graph 1.
Standard Curve



The protein concentration of the unknown sample can then be determined. From the graph above, the protein concentration of the unknown samples (diluted and undiluted) (Tubes 7, 8, and 9) would be 20 μg/ml, 10 μg/ml, and 2 μg/ml, respectively. Accounting for the dilution of the sample for tubes 8 and 9, all three (tubes 7-9) give a value of 20 μg/ml for the protein concentration of the unknown sample.

One can calculate the amount of protein present in each unknown sample used in the assay and the total amount of protein present in the entire unknown sample by using the equations below:

(μg/ml of unknown sample) times
(ml of unknown sample used for assay) times
(Dilution Factor)

Tube 8 - Sample Calculation
(10 μg/ml) × (1 ml) × (2) = 20 μg of protein present in tube 8

To calculate the total amount of protein present in the unknown solution, multiply the concentration of the unknown solution by its total volume. For this example, the total volume of the unknown solution is 30 ml.

(unknown sample concentration [μg/ml]) times
(total volume of unknown solution [ml])

Calculation of protein content of unknown solution
(20 μg/ml) × (30 ml of total unknown solution) = 600 μg of protein present in unknown solution.

B. Custom Assay Protocols

The QuantiPro BCA assay can be adapted for use with a wide variety of smaller volume containers. It can be used in microcentrifuge tubes, 96 well plates, and 384 well plates. These containers can be used as long as five main points remain unchanged:

1. Read the absorbance 562 nm. For a multiwell plate reader, which does not have the exact filter, a filter in the range of 540–590 nm can be substituted (See Graph 2).

2. Keep the ratio of the volumes of the protein sample and QuantiPro Working Reagent at 1:1.
   Examples:

   Microcentrifuge (0.5 ml protein sample to 0.5 ml QuantiPro Working Reagent).

   96 well plate (0.150 ml protein sample to 0.150 ml QuantiPro Working Reagent).

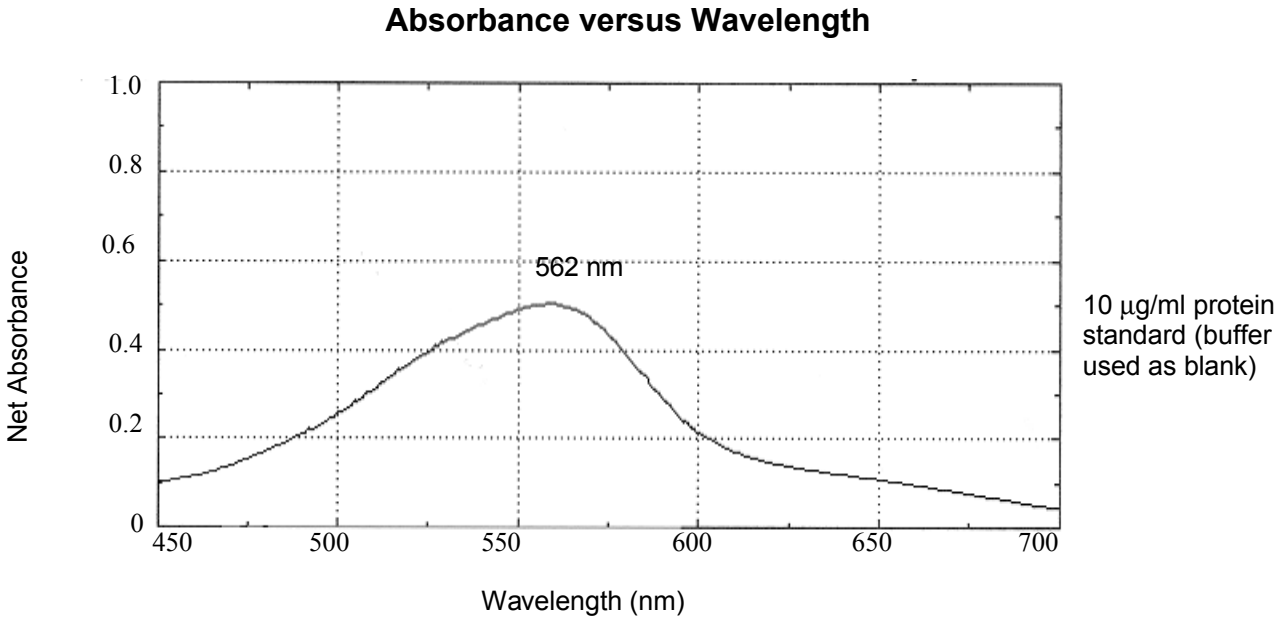
   384 well plate (0.04 ml protein sample to 0.04 ml QuantiPro Working Reagent).
   When using multiwell plates, make sure the unknown sample/standard is present in the well prior to adding the QuantiPro Working Reagent to facilitate mixing.

3. Make sure the protein assay containers are sealed (cover the multiwell plates with a plate sealer) and incubate the samples for:
   60 °C for 1 hour **Or**
   37 °C for 2 hours **Or**
   25 °C (Room Temperature) for 16 hours (overnight).

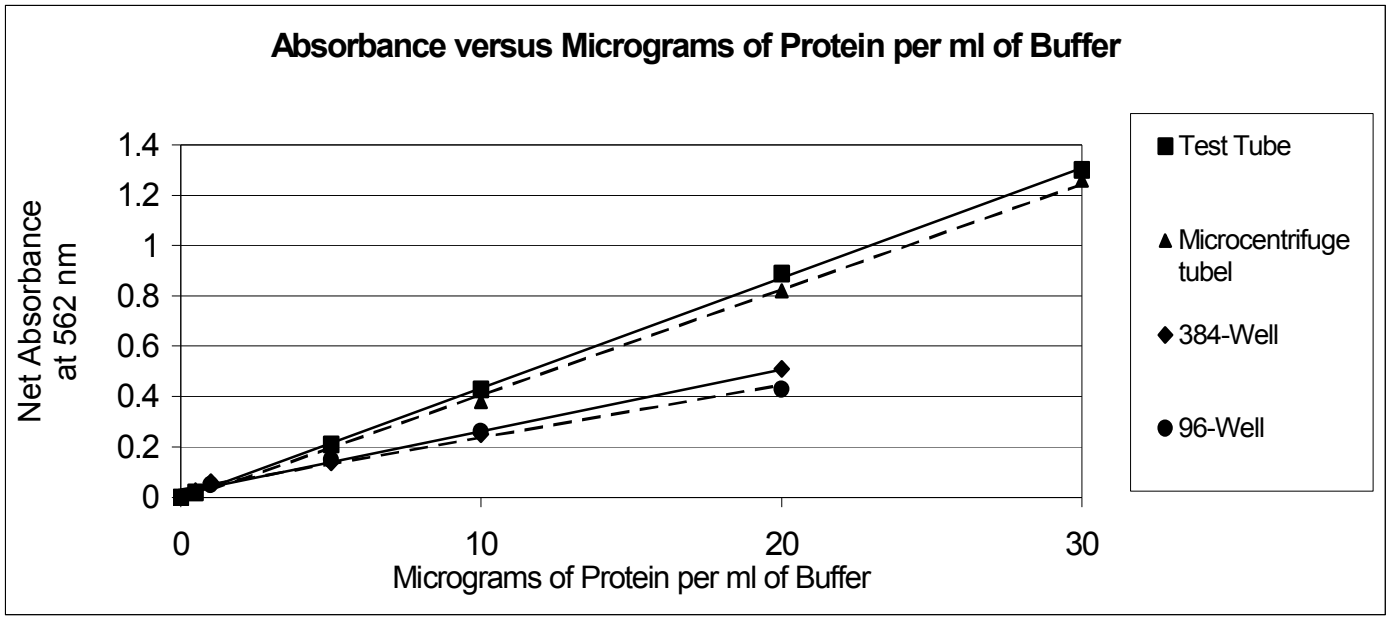
4. Keep the protein sample concentration between 0.5–30 μg/ml for volumes of protein sample greater than 150 μl. For protein samples less than or equal to 150 μl, keep the concentration between 1–20 μg/ml.

5. Separate standard curves will have to be determined for each assay protocol. The pathlength in each assay is dependent on the assay container (cuvettes or multiwell plates) and/or reaction volume. This affects the Net Absorbance values (See Graph 3).

Graph 2.
Wavelength scan (450–700 nm) of a 10 µg/ml protein standard incubated with QuantiPro Working Reagent.



Graph 3.
Typical Standard Curves



All standards were incubated at 60 °C for 1 hour.
Note: Net Absorbance varies with the different pathlength)

C. TCA Concentration-QuantiPro BCA Assay Protocol

By using this procedure it is possible to remove some of the interfering substances that are described in the compatibility chart. It is also possible to increase the concentration of the unknown sample using this procedure.

1. Add the unknown samples and BSA protein standards to separate microcentrifuge tubes and adjust the final volumes to 1.0 ml with deionized water. Larger volumes can also be used by adjusting the following volumes accordingly.
2. Add 0.1 ml of a 0.15% (w/v) solution of sodium deoxycholate (Catalog Number D5670) in deionized water.
3. Mix and let stand for 10 minutes at room temperature. It is also possible to let stand on ice for 10 minutes.
4. Add 0.1 ml of 6.1 N (~100% w/v) solution of trichloroacetic acid (TCA, Catalog Number T0699).
5. Cap and vortex each sample.
6. Incubate for 5 minutes at room temperature. It is also possible to let stand on ice for 5 minutes.
7. Centrifuge the samples for 15 minutes at room temperature in a microcentrifuge at full speed.
8. Carefully decant or pipette the supernatant of each sample. Do not disturb the pellet.
9. Solubilize each pellet by adding 40 µl of a 5% (w/v) solution of sodium dodecyl sulfate (SDS, Catalog Number L6026) prepared using a 0.1 N sodium hydroxide solution. Mix well until pellet is completely dissolved.
10. Pipette 0.96 ml of deionized water into the tube so that the 2 ml standard assay procedure can be performed. It is possible to add less water if a smaller volume assay is to be performed.
11. Vortex each sample and proceed onto the 2 ml standard assay protocol or a custom assay.

Compatibility Chart

The amount listed is the maximum amount of material allowed in the protein sample prior to adding the QuantiPro Working Reagent without causing noticeable interference. Use the TCA concentration method to remove incompatible substances (Procedures, Section III).

Incompatible Substances	Amount Compatible
Buffer SYSTEMS	
N-Acetylglucosamine (10 mM) in PBS, pH 7.2	None – Not Compatible
ACES, pH 7.8	10 mM
Bicine, pH 8.4	2 mM
Bis-Tris, pH 6.5	0.2 mM
CelLytic™ B Reagent	10-fold dilution
CelLytic B II Reagent	20-fold dilution
Calcium chloride in TBS, pH 7.2	10 mM
CHES, pH 9.0	100 mM
Cobalt chloride in TBS, pH 7.2	None – Not Compatible
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	0.5 mM
HEPES	100 mM
MOPS, pH 7.2	100 mM
Nickel chloride in TBS	0.2 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	Undiluted No Interference
PIPES, pH 6.8	100 mM
Sodium acetate, pH 4.8	200 mM
Sodium citrate, pH 4.8 or pH 6.4	5 mM
Tricine, pH 8.0	2.5 mM
Triethanolamine, pH 7.8	0.5 mM
Tris	50 mM
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6 (Catalog Number T5030)	10-fold dilution
Tris (25 mM), Glycine (1.92 M), SDS (0.1%), pH 8.3 (Catalog Number T4904)	10-fold dilution
Zinc chloride (10 mM) in TBS, pH 7.2	0.5 mM
Buffer Additives	
Ammonium sulfate	None – Not Compatible
Aprotinin	1 mg/L
Cesium bicarbonate	100 mM
Glucose	1 mM

Incompatible Substances (Continued)	Amount Compatible
Buffer Additives (Continued)	
Glycerol	1%
Guanidine•HCl	4 M
Hydrochloric acid	10 mM
Imidazole	12 mM
Leupeptin	10 mg/L
PMSF	1 mM
Sodium azide	0.2%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium hydroxide	50 mM
Sodium phosphate	100 mM
Sucrose	4%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Sodium orthovanadate in PBS, pH 7.2	1 mM
Thimerosal	None - Not Compatiible
Urea	3 M
Chelating agents	
EDTA	0.5 mM
Sodium citrate	5 mM
Detergents	
BRIJ® 35	5%
BRIJ 52	1%
CHAPS	5%
CHAPSO	5%
Deoxycholic acid	5%
Lubrol® PX	1%
Nonidet P-40 (NP-40)	5%
Octyl β-glucopyranoside	5%
Octyl β-thioglucopyranoside	5%
SDS	5%
SPAN® 20	1%
Triton™ X-100	5%
Triton X-114	0.05%
Triton X-305	1%
Triton X-405	1%
TWEEN® 20	5%
TWEEN 60	0.5%
TWEEN 80	5%
SB3-10	None - Not Compatible

Incompatible Substances (Continued)	Amount Compatible
Reducing & Thiol Containing Agents	
Dithioerythritol (DTE)	None - Not Compatible
Dithiothreitol (DTT)	None - Not Compatible
2-Mercaptoethanol	1 mM
Tributylphosphine	None - Not Compatible
tris (2-Carboxyethyl)phosphine TCEP	None - Not Compatible
Solvents	
Acetone	1%
Acetonitrile	1%
DMF	1%
DMSO	1%
Ethanol	1%
Methanol	1%

Note: This is not a complete list of incompatible compounds. There are many substances that affect various proteins in different ways. One may assay the protein of interest in deionized water alone, then in the sample buffer with possible interfering substances. Comparison of the readings will indicate if an interference exists. Refer to references below for additional information on interfering substances.[1-9]

Troubleshooting Guide

Standard Curve is not linear.

1. The linearity range normally obtained with this procedure without the presence of interfering substances is 0.5–30 μg/ml. If the standard curve is linear, but starts to curve inside the upper (30 μg/ml) and/or lower (0.5 μg/ml) limits of the linearity range, this may be an indication of an interfering substance in the buffer system being used. It may be necessary to adjust the working linearity range for a given buffer system by lowering the upper limit to 20 μg/ml or raising the lower limit to 2–5 μg/ml. The protein concentration of the unknown samples must fall within the working linearity range for the buffer system used. For samples with higher protein concentrations, the BCA kit (Catalog Number BCA1) may be more appropriate (linearity range of 200–1,000 μg/ml).

2. Another indication of the presence of an interfering substance is a standard curve with a very small slope. In this case it will be necessary to lower the amount of interfering substance present in the buffer system used (see Procedures, Section III).

3. If you are using this assay in a 384 well format, make sure the liquids (protein samples or working reagent) are not sticking to the side of the wells. This is done by making sure the robotic pipetter, if used, is properly calibrated to the position of the plate wells.

Sample Handling to Eliminate Interference by Incompatible Substances.

1. Make sure the glassware being used has been thoroughly cleaned.

2. If the starting protein concentration of the unknown sample is high enough, dilute the sample so the incompatible substance is at a level, which no longer causes interference.

3. Use the TCA Concentration-QuantiPro BCA procedure (Procedures, Section III).

4. Consider a different protein assay procedure. If the presence of certain incompatible reagents (reducing agents) is necessary, consider the use of the Bradford Assay (**Catalog Number** B6916).

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