BCA Protein Assay Kit

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About the Kit

BCA Protein Assay Kit 71285-3

Description

The BCA Protein Assay is based on a biuret reaction, the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline solution, and a concentration-dependent detection of the monovalent copper ions (Cu^{1+}) produced. Bicinchoninic acid is a chromogenic reagent that chelates with the reduced copper, producing a purple reaction complex with strong absorbance at 562 nm (1). The kit can be used to determine protein concentrations in the range of 20–2,000 µg/ml in either the standard assay or microassay size. Kit components are sufficient to complete 500 standard size reactions (50 µl protein sample plus 1 ml reagent) or 2,500 micro-scale reactions (25 µl protein sample plus 200 µl reagent in 96-well plate). For convenient preparation of protein concentration standard curves, a bovine serum albumin (BSA) standard (2 mg/ml) is provided.

The BCA Protein Assay is robust and can be performed in the presence of many chemical compounds and detergents including the Novagen BugBuster®, PopCulture®, CytoBuster™, Reportasol™ and Insect PopCulture® lysis reagents. Some reagents, including chelating agents, strong acids or bases, and reducing agents interfere with the reduction and chelating reactions on which this assay depends.

Components

- 500 ml BCA Solution (bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 M NaOH, pH 11.25)
- 15 ml 4% Cupric Sulfate
- 3 × 1 ml BSA Standard (2 mg/ml)

Storage

Store all components at room temperature.

BCA Protein Assay

Preparation of BSA standards

Table 1 below gives guidelines for preparing diluted BSA standards. Prepare each standard in a clean vial. To ensure the effects of the buffer are taken into consideration, it is recommended that the dilutions are made in the same buffer as the samples. Deionized water may be used instead of the sample buffer, however, any interference from the buffer will not be corrected for in the BSA standard curve.

Table 1 Preparation of BSA Standards

Dilutions for standard assay

Tube	Volume of BSA	Volume of diluent	Final BSA concentration
1	250 µl from 2 mg/ml solution	250 µl	1,000 µg/ml
2	250 µl from tube 1	250 µl	500 µg/ml
3	250 µl from tube 2	250 µl	250 µg/ml
4	300 µl from tube 3	300 µl	125 µg/ml
5	100 µl from tube 4	400 µl	25 µg/ml
6	0	400 µl	0 µg/ml

Dilutions for enhanced assay

Tube	Volume of BSA	Volume of diluent	Final BSA concentration
1	100 µl from 2 mg/ml solution	700 µl	250 µg/ml
2	400 µl from tube 1	400 µl	125 µg/ml
3	300 µl from tube 2	450 µl	50 µg/ml
4	200 µl from tube 3	200 µl	25 µg/ml
5	100 µl from tube 4	400 µl	5 µg/ml
6	0	400 µl	0 µg/ml

Preparation of BCA working reagent

Table 2 below gives guidelines for preparing the BCA working reagent for both the test tube and microtiter assays. Working reagent is prepared by mixing 50 parts BCA solution with 1 part of 4% Cupric Sulfate.

Note: The volume of BCA working reagent for the test tube assay is based on 1 ml of reagent per reaction. This volume is sufficient if reading the absorbance in a microcuvette (0.7 ml). If the cuvette requires a larger minimum volume, up to 3 ml of reagent may be need per reaction.

Table 2 Preparation of BCA working reagent

	For each sample	× 20
Test tube assay		
BCA Solution	1 ml	20 ml
4% Cupric Sulfate	20 µl	400 µl
Micro-scale assay		
BCA Solution	200 µl	4 ml
4% Cupric Sulfate	4 µl	80 µl

Assay protocols

The following procedures describe two assay protocols. The test tube assay requires a larger volume of protein sample (50 µl). However, the effect of interfering substances is minimized because the ratio of BCA working reagent to protein sample is 20:1. The micro-scale assay requires less of the protein sample (25 µl) and offers the convenience of a 96-well plate. However, because the ratio of BCA working reagent to protein sample is 8:1 the effect of interfering substances will be greater.

Test tube assay

1. Pipette 50 µl each standard or protein sample replicates into labeled test tubes.
2. Add 1.0 ml BCA working reagent. Mix by gentle vortexing.

   Note: The volume of the reaction may need to be increased to 3.15 ml (3 ml BCA working reagent plus 150 µl standard or protein sample) if the cuvette requires a larger minimum volume for measuring the absorbance.
3. **Standard assay**: Incubate reactions at 37°C for 30 min or at room temperature for 2–16 h.
   Enhanced assay: Incubate reactions at 60°C for 15 min.
4. Allow tubes to cool to room temperature.

   Note: Color development continues after cooling to room temperature. However, no significant error in protein concentration is observed when all the reactions are read within 10 min.
5. Add 1 ml water to a clean cuvette and adjust the absorbance reading at 562 nm to zero.
6. Transfer the reactions to clean cuvettes.
7. Measure and record the absorbance (A562) of all reactions within 10 min.
8. To obtain the corrected absorbances, subtract the absorbance of the blank standard from the absorbance measurement of all other standard and protein samples.
9. Plot the corrected absorbance versus the known mass of the BSA standards to generate the standard curve.
10. Using the standard curve, interpolate the recorded corrected absorbance reading for the samples assayed which fall within the linear range of the standard curve.
11. Calculate the amount of protein present in the original sample by correcting for the dilution and sample volume.

Micro-scale assay

1. Pipette 25 µl each standard or protein sample replicate into individual wells of a 96-well plate.
2. Add 200 µl BCA working reagent to each well. Mix on plate shaker for 30 seconds. Cover plate.
3. **Standard assay:** Incubate reactions at 37°C for 30 min or at room temperature for 2–16 h.
   Enhanced assay: Incubate reactions at 60°C for 15 min.
4. Cool plate to room temperature.
5. Measure the absorbance at 562 nm on a plate reader.

   Note: If the plate reader does not have a 562 nm filter, wavelengths from 540–590 nm have been used successfully.
6. To obtain the corrected absorbances, subtract the absorbance of the blank standard from the absorbance measurement or all other standard and protein samples.
7. Plot the corrected absorbance versus the known mass of the BSA standards to generate the standard curve.

   Note: If a curve-fitting algorithm associated with the microplate reader is used for preparing the standard curve, a four-parameter (quadratic) or best-fit curve will provide the most accurate results.
8. Using the standard curve, interpolate the recorded corrected absorbance reading for the samples assayed which fall within the linear range of the standard curve.
9. Calculate the amount of protein present in the original sample by correcting for the dilution and sample volume.

Troubleshooting

Symptom	Possible cause	Solution
No color development in any of the reactions	Sample contains a copper chelating agent	Dialyze, desalt, or dilute protein sample. Increase copper concentration in BCA working reagent (i.e., use a 50:2 ratio of BCA solution to 4% Cupric Sulfate).
All reactions, including blank are dark purple	Buffer contains a thiol	Dialyze or dilute sample.
	Buffer contains a reducing agent	Dialyze or dilute sample.
	Buffer contains biogenic amines (catecholamines)	Dialyze or dilute sample.
Blank absorbance is OK, but standards and samples absorbance measurements are lower than expected	Absorbance measured at incorrect wavelength	Measure absorbance at 562 nm. Absorbance may be measured between 540–590 nm, although the sensitivity will be reduced.
	Sample is in a strong acid or alkaline buffer	Dialyze, desalt, or dilute sample.
Absorbance measurements of protein samples higher than expected	Sample contains lipids or lipoproteins	Add SDS to the protein sample (final concentration 2%) to reduce interference from lipids (2).
	Protein concentration is too high	Dilute sample.

Substance Compatibility

Incompatible substances

The following substances have been found to interfere with the BCA Protein Assay Kit at small concentrations and should be avoided as components of the sample buffer. If interfering substances cannot be removed, consider using the Non-Interfering Protein Assay™ Kit (Cat. No. 488250) for protein concentration determination. This table is not comprehensive, and it is not certain whether other substances may interfere with the assay.

Asorbic acid	EGTA	Iron	Sucrose (impure)
Catecholamines	Glycerol (impure)	Lipids	Tryptophan
Creatinine	Hydrogen peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric acid

Compatible substances

The following table lists substances that are compatible with the BCA Protein Assay Kit and the maximal non-interfering concentration. This table is not comprehensive, and it is not certain whether other substances may interfere with the assay.

Substance	Concentration
ACES, pH 7.8	25 mM
Acetone	10%
Acetonitrile	10%
Ammonium sulfate	1.5 M
Aprotinin	10 mg/l
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
Borate, pH 8.5	50 mM
Brij®-35	5%
Brij-56, Brij-58	1%
BugBuster® Protein Extraction Reagent (Cat. No. 70584)	undiluted
Calcium chloride (in TBS, pH 8.0)	10 mM
Cesium bicarbonate	100 mM
CHAPS	5%
CHAPSO	5%
CHES, pH 9.0	100 mM
Cobalt chloride (in TBS, pH 8.0)	0.8 mM
CytoBuster™ Protein Extraction Reagent (Cat. No. 71009)	undiluted
Deoxycholic acid	5%
DMF	10%
DMSO	10%
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	0.5 mM
EDTA	10 mM
EPPS, pH 8.0	100 mM
Ethanol	10%
Ferric chloride (in TBS, pH 8.0)	10 mM
Glucose	10 mM
Glycine-HCl, pH 2.8	100 mM

Substance	Concentration
Glycercol (pure)	10%
Guanidine-HCl	4 M
HEPES	100 mM
Hydrochloric acid	100 mM
Imidazole, pH 7.0	50 mM
Insect PopCulture® Reagent (Cat. No. 71187)	undiluted
Leupeptin	10 mg/l
2-Mercaptoethanol	0.01%
MES, pH 6.1	100 mM
Methanol	10%
MOPS, pH 7.2	100 mM
Nickel chloride (in TBS, pH 8.0)	10 mM
Nonidet P-40 (NP-40)	5%
Octyl β-glucoside	5%
Octyl β-thioglucopyranoside	5%
PIPES, pH 6.8	100 mM
PMSF	1 mM
PopCulture Reagent (Cat. No. 71092)	undiluted
Potassium thiocyanate	3.0 M
Reportasol™ Extraction Buffer (Cat. No. 70909)	undiluted
SDS	5%
Sodium acetate, pH 4.8	200 mM
Sodium azide	0.2%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium hydroxide	100 mM
Sodium phosphate	100 mM
Sucrose	40%

Substance (continued)	Concentration
TBS (150 mM NaCl, 100 mM Tris-HCl, pH 8.0)	undiluted
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
Tris(hydroxypropyl)phosphine (THP)	1 mM
Triton® X-100	5%
Triton X-114, X-305, X-405	1%
Tween® 20, Tween 60, Tween 80	5%
Zinc chloride (in TBS, pH 8.0)	10 mM
Zwittergent® 3-14	1%

References

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