CYANAGEN

Reagents for Molecular Biology

THE RIGHT LIGHT

µQPRO

protein quantitation

Bicinchoninic Acid (BCA) kit

02

www.cyanagen.com

About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development and production of reagents for molecular diagnostic since 2003 and one of the leading companies in the field of reagents for Western blotting and Elisa.
The main product lines are focused on chemiluminescence and fluorescent dyes for biological analysis, genomics, proteomics and chemical sensors.
They are based on Cyanagen internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
The products are extremely versatile and perfectly suited to the latest analytical instrumentation. These products are also available as OEM.

Cyanagen s.r.l. has a certified Quality System

ISO 9001-2008 QUALITY CERTIFIED

Product manual

µQPRO

0.5÷20 µg/ml protein

protein quantitation

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µQPRO INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.

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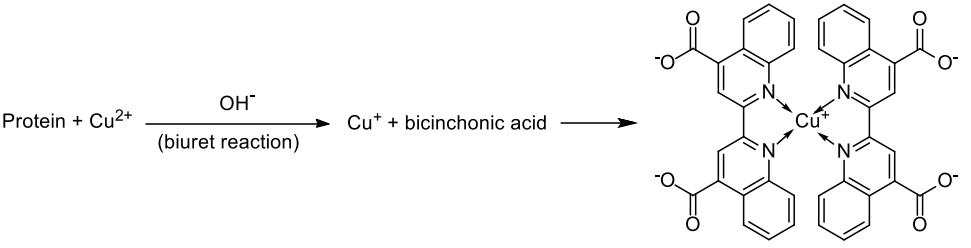
1. Introduction

The μQPro Bicinchoninic Acid Kit for Protein Determination is formulated for the colorimetric detection and quantitation of total protein in the 0.5–20 μg/mL concentration range. The Bicinchoninic Acid (BCA) protein assay, also known as the Smith Assay, is a highly sensitive colorimetric assay that is compatible with detergent solubilized protein solutions. The principle of the bicinchoninic acid (BCA) assay is similar to the Lowry procedure, in that both rely on the formation of a Cu2+-protein complex under alkaline conditions, followed by reduction of the Cu2+ to Cu1+, in a temperature dependent reaction. The amount of Cu2+ reduced is proportional to the amount of protein present in the solution. Next, two molecules of bicinchoninic acid (BCA) chelate with each Cu+ ion, forming a purple-colored product that strongly absorbs light at a wavelength of 562 nm that is linear for increasing protein. The amount of protein present in a solution can be quantified by measuring the absorption spectra and comparing with protein solutions with known concentrations.



Purple Complex of Cu^{+} with BCA

Cu(BCA)2 complex has a strong molar extinction coefficients at 592 nm of 7 700 L M-1cm-1

The BCA method is faster and easier than Lowry method, eliminating the need for precisely timed reagent additions and vortexing inherent, providing enhanced flexibility and ease of use with much greater tolerance to interference from non-ionic detergents and buffer salts. In particular, it is insensitive to detergents such as Triton-100 and SDS (1%).

References:

1. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., *Anal. Biochem.* 1985; 150:76-85.
2. Walker, J.M., *Methods Mol. Biol.* 1994; 32:5-8.
3. Pingoud A., Urbanke, C, Hoggett J. Jeltsch, A., Biochemical Methods, pp. 157-159. Wiley-VCH 2002.

Storage

Store at Room Temperature

2. Important notes

a) Precipitates, which may form in Reagent A, or B during storage should be dissolved by gentle warming and stirring.
b) Color development continues even at room temperature. However, no significant error is introduced if all absorbance measurements are completed within 10 minutes.
c) Reagents that chelate metal ions, change the pH of the assay or reduce copper are known to interfere with the assay. Please check that the following components are not in the sample buffer: ascorbic acid, catecholamines, creatinine, cysteine, EGTA, impure glycerol, hydrogen peroxide, hydrazides, iron, lipids, melibiose, phenol red, impure sucrose, tryptophan, tyrosine, uric acid.
d) Other substances affect the assay to a lesser extent and, if their concentration in the sample buffer is below a certain value, they can be tolerated. Please refer to Compatibility Chart for maximum compatible concentrations of many of these substances.
e) It is necessary to create a standard curve during each assay regardless of the format used. μQPro working solution is stable for several days at RT. If not used immediately, it should be stored at RT in a closed container.

3. Components and other materials required

Kit components

Kit includes reagents sufficient for 480 test tubes or 3,200 microplate assays.

- Reagent A (tartrate in carbonate buffer) 240 ml
- Reagent B (4% BCA in water) 240 mL
- Reagent C (4% $CuSO_4 \cdot 5H_2O$ in water) 12 mL

Other required equipment/materials

- Spectrophotometer capable of measuring absorbance at 560 nm
- Water bath
- Test tubes or 96 well plate
- Protein Standards

4. Preparation of the Working Reagent (WR)

Mix 25 Parts of Reagent A and 24 parts of Reagent B with 1 part of Reagent C.

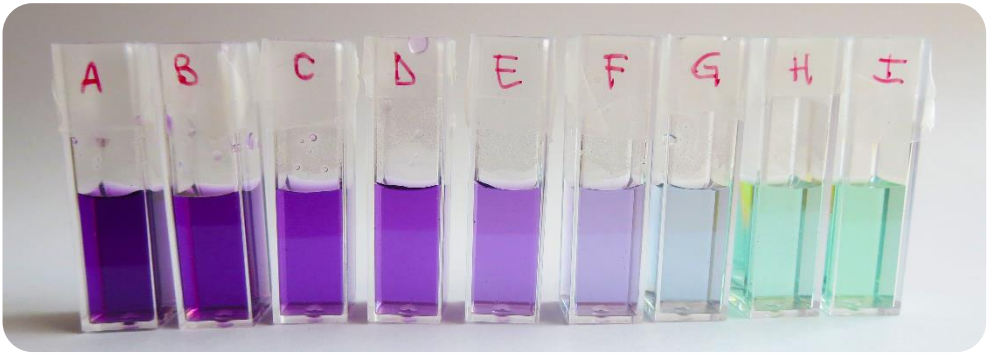
The amount of Working Reagent required for each sample is:

- 1 mL for the Test Tube Procedure
- 150 µL for the Micro-assay Plate Procedure

Prepare sufficient volume of WR for the samples to be assayed plus the calibration standards. For accurate and reproducible results always prepare fresh WR.

5. Preparation of the Calibration Standards

Prepare a fresh set of protein standards in the 0.5-200 µg/mL range, preferably using the same diluent as your sample, starting from a **200 µg/mL stock solution of Bovine Serum Albumin Standard (BSA).**



Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	4.5mL	0.5mL of Stock 200 µg/mL	200 µg/mL
B	8.0mL	2.0mL of vial A dilution	40µg/mL
C	4.0mL	4.0mL of vial B dilution	20µg/mL
D	4.0mL	4.0mL of vial C dilution	10µg/mL
E	4.0mL	4.0mL of vial D dilution	5µg/mL
F	4.0mL	4.0mL of vial E dilution	2.5µg/mL
G	4.8mL	3.2mL of vial F dilution	1µg/mL
H	4.0mL	4.0mL of vial G dilution	0.5µg/mL
I	8.0mL	0ml	0µg/mL = Blank

Correction Factors relative to BSA are reported in the following Table:

Protein	Correction Factor
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.80
α-Chymotrypsinogen, bovine	0.99
Cytochrome C, horse heart	1.11
Gamma globulin, bovine	0.95
IgG, bovine	1.12
IgG, human	1.03
IgG, mouse	1.23
IgG, rabbit	1.12
IgG, sheep	1.14
Insulin, bovine pancreas	1.22
Myoglobin, horse heart	0.92
Ovalbumin	1.08
Transferrin, human	0.98

avg. "test" net Abs. = Correction Factor * avg. BSA net Abs.

NOTE: When preparing the standard curve if using curve-fitting algorithms a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

6. Test Tube Procedure (linear working range of 0.5-20µg/mL)

1. Pipette 1.0mL of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 1.0mL of the WR to each tube and mix well.
3. Cover tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. With the spectrophotometer set at 562nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in µg/mL.
8. Use the standard curve to determine the protein concentration of each unknown sample.

NOTE: Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562nm absorbance measurements of all tubes are made within 10 minutes of each other.

7. Microplate Procedure (linear working range of 2-40µg/mL)

1. Pipette 150µL of each standard or unknown sample replicate into a microplate well.
2. Add 150µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate using Sealing Tape for 96-Well Plates and incubate at 37°C for 2 hours.
4. Cool plate to room temperature (RT).
5. Measure the absorbance at or near 562nm on a plate reader.
6. Subtract the average 562nm absorbance reading of the Blank standard replicates from the 562nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in µg/mL.
8. Use the standard curve to determine the protein concentration of each unknown sample.

NOTE: Limit incubations of microplate to less than or equal to 37°C, otherwise high background and aberrant color development may result. Most polystyrene assay plates deform at 60°C.

8. Methods for eliminating/reducing the effect of interfering substances

- Remove the interfering substance by dialysis or gel filtration
- Dilute sample. This works only if the starting protein is sufficiently concentrated to remain in the working range upon dilution
- Precipitate the proteins in the sample with acetone or trichloroacetic acid. The protein pellet is then solubilized in ultrapure water or directly in the Working Reagent.
- Increase the amount of copper in the Working Reagent (e.g. use a greater proportion of Reagent C; e.g. Reagent A:B:C equal 25:24:2 or 25:24:3).

NOTE: For best accuracy, the protein standards must be treated identically to the sample(s).

9. Compatibility Chart

NOTE: * Diluted with ultrapure water. NC: not compatible.

Detergents	**Concentration**
Brij®-35	5.0%
Brij®-56, Brij®-58	1.0%
CHAPS (CHAPSO)	1.0% (5.0%)
Deoxycholic acid	5.0%
Nonidet P-40 (NP-40)	5.0%
Octyl ß-glucoside	0.1%
Octyl ß -thioglucopyranoside	5.0%
SDS	5.0%
Span® 20	1.0%
Triton® X-100	5.0%
Triton® X-114	0.05%
Triton® X-305, X-405	1.0%
Tween®-20, Tween®-80	5.0%
Tween®-60	0.5%
Zwittergent® 3-14	NC

Chelating agents	**Concentration**
EDTA	0.5 mM
EGTA	NC
Sodium citrate, pH 4.8 (or pH 6.4)	5 mM (16.7mM)

Reducing & Thiol Containing Agents	**Concentration**
N-acetylglucosamine in PBS	NC
Ascorbic acid	NC
Cysteine	NC
Dithioerythritol (DTE)	NC
Dithiothreitol (DTT)	NC
Glucose	1 mM
2-Mercaptoethanol	1 mM
Thimerosal	NC

Salts/Buffers	Concentration
ACES, pH 7.8	10 mM
Ammonium sulfate	NC
Bicine, pH 8.4	2 mM
Bis-TRIS, pH 6.5	0.2 mM
Calcium chloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6 M),Na-Carbonate (0.1 M), pH 9.0	1:600 dilution*
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5	1:600 dilution*
Cobalt chloride in TBS, pH 7.2	NC
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	0.5 mM
Glycine•HCl, pH 2.8	100 mM
Guanidine•HCl	4 M
HEPES, pH 7.5	100 mM
Imidazole, pH 7.0	12.5 mM
MES, pH 6.1	100 mM
MES (0.1 M), NaCl (0.9%), pH 4.7	1:4 dilution*
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4	undiluted
Nickel chloride in TBS, pH 7.2	0.2 mM
PBS; Phosphate (0.1 M),NaCl (0.15 M) pH 7.2	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer; 50 mM TRIS, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1:10 dilution*
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)	5 mM (16.7 mM)
Sodium phosphate	100 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	1:10 dilution*
TRIS (25 mM), Glycine (192 mM), pH 8.0	1:10 dilution*
TRIS (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3	undiluted
Zinc chloride in TBS, pH 7.2	0.5 mM

Misc. Reagents & Solvents	Concentration
Acetone	1.0%
Acetonitrile	1.0%
Aprotinin	1 mg/L
DMF, DMSO	1.0%
Ethanol	1.0%
Glycerol (Fresh)	1.0%
Hydrazide	NC
Hydrides (Na2BH4 or NaCNBH3)	NC
Hydrochloric Acid	10 mM
Leupeptin	10 mg/L
Methanol	1.0%
Phenol Red	NC
PMSF	1 mM
Sodium Hydroxide	50 mM
Sucrose	4%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea	3 M
o-Vanadate (sodium salt), in PBS, pH 7.2	1 mM

10. Troubleshooting

No color development

Possible Cause	Precautions/Remedies
Chelating agents are present in the sample buffer	Dialyze or desalt the sample. Dilute the sample

Sample color less intense than expected

Possible Cause	Precautions/Remedies
pH is altered by strong acid or alkaline buffer	Dialyze or desalt the sample. Dilute the sample

Sample color is darker than expected

Possible Cause	Precautions/Remedies
Protein concentration is too high	Dilute the sample
Lipids or lipoproteins are present in the sample buffer	Add 2% SDS to the sample to eliminate interference from lipids

All the tubes are dark purple

Possible Cause	Precautions/Remedies
Reducing agents are present in the sample buffer	Dialyze or dilute the sample
Thiols are present in the sample buffer	Dialyze or dilute the sample

11. Ordering information

Product Description:	Quantity:	Sufficient For:	Order-No:
QPRO - BCA kit Standard 20÷2000 μg/ml protein	500 mL of 1% BCA/tartrate in alkaline carbonate buffer (Solution A) and 15 mL of 4% $CuSO_4$ $5H_2O$ in water (Solution B)	250 test tube or 2500 microplate	PRTD1,0500
μ QPRO - BCA kit Micro 0.5÷20 μg/ml protein	240 mL of tartrate in alkaline carbonate buffer (Reagent A), 240 mL of 4% BCA in water (Reagent B) and 12 mL of 4% $CuSO_4$ $5H_2O$ in water (Reagent C)	480 test tube or 3200 microplate	PRTD2,0500

For further information, visit **www.cyanagen.com**

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