FT-40840A

BC Assay : Protein Quantitation Kit

Product Description

High quality reagents for the determination of protein concentration by the bicinchoninic acid method

Product Number	Product Name and Description
UP40840A	BC Assay Protein Quantitation Kit, sufficient reagents for 500 test-tube or 5 000 microplate assays
	Contains: UP95424A, reagent A, 1000ml
	UP95425A, reagent B, 25 ml
	UP36859A, Albumin Standard, 2 mg/ml, 10x1 ml
UP40840B	BC Assay Protein Quantitation Kit, sufficient reagents for 125 test-tube or 1 250 microplate assays
	Contains: UP95424B, reagent A, 250 ml
	UP95425B, reagent B, 6 ml
	UP36859A, Albumin Standard, 2 mg/ml, 3x1 ml

Storage: On receipt store at room temperature (18 months, long term storage at + 4°C) (Z')

For laboratory use only, not for drug, household or medical use

Scientific and Technical Information

Proteins have traditionally been quantified by the spectrometric measurement of a color produced by a reaction between proteins and a reagent. The 1980's saw the introduction by Paul Smith(a)l of a colorimetric protein assay derived on the Biuret reaction using the bicinchoninic acid.
Our improved formulation, the BC Assay, provides significant benefits over existing colorimetric assays, namely sensitivity, reagent stability, and detergent compatibility.

Principle:
The BC Assay is a colorimetric assay: it involves the reduction of Cu2+ to Cu+ by proteins in an alkaline medium. The BC Assay (bicinchoninic acid) chelates Cu+ ions with very high specificity to form a water soluble purple coloured complex.

Peptide bonds + Cu2+ -----([tetradente- Cu+ complex]
OH- Cu+ + 2 BC Assay ([purple-colored Cu+-BC Assay-Complex]
The reaction should be read at a defined time and temperature condition as the purple colored copper BC assay complex will continually develop. However, the procedural development is slow enough to allow the processing of numerous samples.
The reaction is measured by the high optical absorbance of the final Cu+ complex at 562 nm. Absorbance is directly proportional to the protein concentration, with a broad linear range between 20 – 2000µg/ml and 5 - 250 µg/ml (enhanced protocol). Protein concentration is calculated with a reference curve obtained for a standard protein.

Features of Uptima BC Assay : high quality, optimized, formulated reagents

- High Performance — High sensitivity and wide linear dynamic range:
  20 µg/ml to 2 mg/ml , and 5 µg/ml to 250 µg/ml using the enhanced protocol
  Less protein-protein variation than Coomassie
- Convenient — Very quick protocol : mix the 2 reagents, it is ready to add to samples !
  Stable at room temperature, 18month validity
  Different catalog packages available (plus enquire for custom packages)
- Applications — Determination of protein concentration in complex mixtures,
  particularly suited to complex mixtures containing nucleic acids, lipids or detergents
  Adaptable to immobilized proteins (coated plates, affinity gels…)
  Adaptable to quantitate Cu+

Assay Procedures

Labware must be carefully cleaned and rinsed with distilled water to avoid traces of interfering substances such as proteins and metals.

Preparation of standards :
Uptima recommends to use the albumin standard (#UP36859A, BSA at 2 mg/ml) for most applications.
Assayed proteins should be utilized as the standard if greater accuracy is required.

Prepare a fresh set of protein standards at 2 mg/ml to 20 µg/ml, diluted from the stock solution in the same buffer, or alternatively in water (in this instance, the sample buffer(s) should be checked by analysis versus water).

Standard protocol (20 µg/ml-2 mg/ml)

Standard	BSA standard	Water or Buffer	Final protein Concentration
Stock Solution	BSA 2 mg/ml #UP36859A		
Standard A	300 µl of stock	0 µl	2 mg/ml
Standard B	300 µl of stock	300 µl	1 mg/ml
Standard C	420 µl of stock	700 µl	750 µg/ml
Standard D	100 µl of stock	300 µl	500 µg/ml
Standard E	100 µl of stock	700 µl	250 µg/ml
Standard F	200 µl of (E)	300 µl	100 µg/ml
Standard G	100 µl of (E)	400 µl	20 µg/ml
Blank H	0	700 µl	0

Enhanced protocol (5 µg/ml-250 µg/ml)

Standard	BSA standard	Water or Buffer	Final protein Concentration
Stock Solution	BSA 2 mg/ml #UP36859A		
Standard A'	100 µl of stock	300 µl	500 µg/ml
Standard B'	100 µl of stock	700 µl	250 µg/ml
Standard C'	300 µl of (B')	300 µl	125 µg/ml
Standard D'	100 µl of (B')	400 µl	50 µg/ml
Standard E'	48 µl of (B')	752 µl	15 µg/ml
Standard F'	16 µl of (B')	784 µl	5 µg/ml
Standard G'	6 µl of (B')	746 µl	2 µg/ml
Blank H'	0	800 µl	0

Preparation of samples:
The protein concentration must fall in the range of standard curve. Therefore it may be useful to prepare several dilutions to meet this requirement.
Dilute samples if necessary with their respective buffer or water.
Sample buffers should be assayed alone to control potential interference.

-Label the tubes and record dilution factor. For example :

Sample *(name)*	Volume sample	Buffer (or water)	Dilution	OD at 562 nm	Assayed Protein Concentration (1)	Protein Concentration in Sample (2)
#1: *sample1*	*200*	*0*	*1*	*0.824*	*0.8 mg/ml*	*0.8 mg/ml*
#2: *sample2*	*20 µl*	*180 µl*	*1/10*	*0.49*	*0.405 mg/ml*	*4.05 mg /ml*
#3: *sample2 dialysed*	*40 µl*	*160 µl*	*1/5*			
#4: *sample3*						

(1) Protein concentration = calculated from OD at 562nm with the standard curve
(2) Protein concentration in sample = assayed protein concentration X dilution factor

Preparation of the BC Assay reagent (A+B mix at 50:1):
Prepare the BC Assay Working Reagent (WR) by adding 1 part of reagent B to 50 parts of reagent A.
Use the table below to determine the required volume of WR

Following is a table for the preparation of the volume of reagent needed with the recommended 8 points standard curve:

Tube assay (Uniplicates)*

Number of		Reagent A	Reagent B
standards	sample		
8 points	1 to 2	20 ml	400 µl
	10 to 12	40 ml	800 µl
	20 to 22	60 ml	1.2 ml

Microplate assay (Duplicates)**

Number of		Reagent A	Reagent B
standards	sample		
8 points	8 to 16	10 ml	200 µl
	32 to 40	20 ml	400 µl
	60 to 64	30 ml	600 µl

* A duplicate analysis is recommanded for accurate determination (even triplicates **)

Use the mixed BC Assay reagent in the next few hours.
Although the Working Reagent can be stored appropriately for a few days, Uptima recommends it is disposed of to avoid potential contamination or degradation, which may affect analysis.

Test Tube Assay Protocols:

	Standard protocol Working range 20µg/ml to 1-2mg/ml	**No heating protocol** Working range 20µg/ml to 1-2mg/ml	**Enhanced Protocol** Working range 5-250 µg/ml
	Allow the reagent to reach room temperature		
a	Pipet 0.1 ml of each standard, control, and sample into test tubes. Duplicates are recommended.		
b	Add 2 ml of BC Assay Working Reagent (mix A+B at 50:1) per test tube, and mix		
c	Incubate		
	at +37°C for 30 min	2 hours at Room Temperature	30 min at +60°C
d	Cool all test tubes to room temperature and measure the optical absorbance (OD) at 562 nm against the blank (water, or buffer + BC Assay reagent).		
e	Plot the standard curve, and interpolate the protein concentration in sample from ODs .		

Microplate Assay Protocols:

	Standard protocol Working range 20µg/ml to 1-2mg/ml	**No heating protocol** Working range 20µg/ml to 1-2mg/ml	**Enhanced Protocol** Working range 5-250 µg/ml
	Allow the reagent to reach room temperature		
a	Pipet 25 µl of each standard, control, and sample into microplates wells. Duplicates or triplicates are recommended		
b	Add 200 µl of BC Assay working reagent (mix A+B at 50:1) per test well, and mix (be careful with cross-contamination)		
c	Incubate		
	at +37°C for 30 min	2 hours at Room Temperature	30 min at +60°C
d	Right after, cool all the microplate to room temperature and read the optical absorbance (OD) at 562 nm against the blank (water, or buffer + BC Assay reagent). Alternatively, wavelengths from 540 to 590 nm have been used.		
e	Plot the standard curve, and interpolate the protein concentration in sample from ODs (see below figure 1).		

Directions for Use

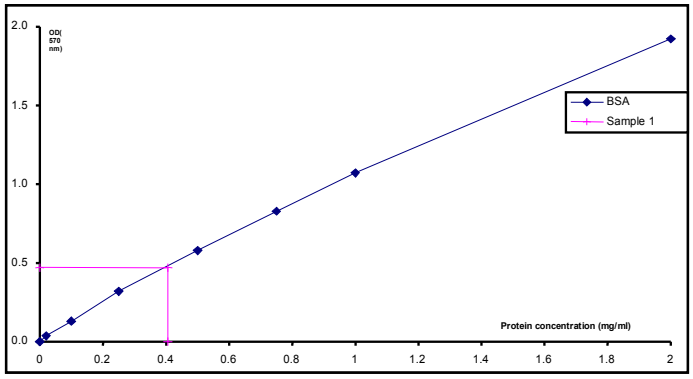
Protocols :

The suggested protocols are convenient and appropriate for the majority of applications.
Modified protocols may may be adopted to suit specific custom requirements. However, clients are recommended to take caution when adopting such procedures as this may affect other performances. i.e.,
-increasing the volume ratio of sample to reagent (i.e.50µl sample instead of 25µl in microplate procedure) increases sensitivity in terms of protein concentration, but may decrease the working range and increase interferences. Lowering the ratio may be helpful to reduce interferences (but sensitivity is decreased).
-reading the absorbance can be done between 540 and 590 nm, for example with microplate readers lacking a 562 nm filter. Absorbances, hence the sensitivity, is however reduced.
-Raising temperature may increase the absorbances values, both signal and background. [1min microwave protocol available (NT)].
-Increasing the time of incubation has a similar effect. This may be useful when reading absorbance at non-optimal wave length

Protein Standard :

Uptima protein assay kits (#UP40840) includes the Bovine Serum Albumin #UP36859. BSA is a common standard that works for most applications (see below the standard curve).Certain applications may benefit for the inclusion, within the analysis, of other purified proteins or even any known sample (e.g. the extract of a reference protein).

The diagram highlights a typical standard curve with bovine albumin standard # UP36859



However, protein to protein variations may occur with some degree depending on several parameters :
. amino-acid sequences rich in cysteine, tryptophane, tyrosine may increase the BC Assay reaction
. the colored response may be affected by the primary structure (sequence order), secondary and tertiary (steric conformation) structures of the protein, isoelectric point (pI), side chains, prosthetic groups…
BC Assay can quantitate immobilized proteins (e), for example gel-coupled proteins, or cells adhering to plates.

Compatible and Interfering substances
One of the principal benefits of the BC Assay is it's compatibility with a lot of substances, notably detergents[(b)] and lipids which can interfere with other colorimetric assays such as Coomassie. The following table highlights some substances and their compatibility.

Compatible (*) Substances	
Detergents	
< 7.5%	CHAPS
< 5%	Deoxycholic acid
< 5%	SDS
< 3%	Triton X-100
< 6%	Tween 20
Solvents	
< 20%	DMSO
< 10%	DMF
< 1 mM	EDTA
< 10%	Glycerol
< 15%	Methanol
< 1 mM	PMSF
< 6 M	Urea
Reducing and thiols containing agents	
< 10 mM	Glucose
< 0.125 mM	DTT

Compatible (*) Substances	
Others	
pure	Carbonate / Bicarbonate 100 mM
< 3 mM	Glycine HCl pH2.8
< 2 M	Guanidine HCl
< 50 mM	Imidazole pH7.0
< 150 mM	MOPS pH7.2
< 1%	NaN_3
pure	PBS (0.1 M phosphate, 150 mM NaCl, pH 7.2)
pure	TBS (20 mM Tris, 150 mM NaCl, pH 7.6)
< 0.01%	Thimerosal
< 50 mM	TRIS
Incompatible Substances	
	Creatinin, Cystein, Tyrosin, Tryptophan
	Mercaptans
	Ascorbic acid, H2O2, hydrazides
	EGTA
	Phenol Red
	Iron, Copper salts

* A substance is classified as 'compatible' if its presence (at a given concentration) does not affect, or changes by less that 10% , the signal of BSA standard at 1 mg/ml. Compatibility may vary depending upon the nature and concentration of the protein being assayed.

Labware may bear traces of metals that affect the BC Assay reaction. Use cleaned or disposable vials.

To limit the interference of some substances ([b], [c], [d]):
-the samples can be diluted provided the protein concentration remains sufficient
-the interfering substance can be removed prior to performing the assay ([f]), i.e. by desalting (dialysis…), precipitation (TCA…), purification…
-increase the A:B ratio of BC Assay reagent up to 20:1 (v/v), to overcome the presence of copper chelators
In such instances, all standards, blanks and controls must be treated in the same way to preserve the accuracy of the assay.

Protein-to-Protein variations
Uptima BC Assay shows far lower Protein-to-Protein variations of signal than Coomassie, and lower than Lowry and most other colorimetric or fluorescent methods. However, several parameters can create, to greater or lesser extents, Protein-to-Protein variation. i.e. Amino-Acid sequences rich in cysteine, cystine, tryptophan, and tyrosine may increase the color development of the BC Assay reaction. Color response may be affected by the primary structure (sequence order), secondary and tertiary structures of the protein, isoelectric point (pI), side chains, and prosthetic groups…

BC Assay can quantitate immobilized proteins([e]). e.g. gel-coupled proteins, or cells adhering to plates.

Other Information

Any questions regarding the use of this product should be directed to Uptima.

Literature

(a) Smith P et al, 1995, Measurment of protein using bicinchiconic acid, Anal. Biochem. 150, 76-85
(b) Kaushal et al, 1986, Effect of Zwitterionic buffers on measurement of small masses of protein with bicinchiconic acid, Anal.Biochem, 157, 291-294
(c) Hill et al, 1988, Protein determination using bicinchiconic acid in the presence of sulfhydryl reagents, Anal.Biochem. 170, 203-208
(d) Kessler R & Fanestil D, 1986, Interference by lipids in the determination of protein using bicinchiconic acid, Anal.biochem.159, 138-142
(e) Stich T, 1990, Determination of protein covalently bound to agarose supports using bicinchiconic acid, Anal.biochem.191. 343-346
(f) Brown et al, 1989, Protein measurement using bicinchiconic acid: elimination of interfering substances. Anal.biochem.180, 136-139