FT-36858A

MicroBC Assay : Protein Quantitation Kit

Product Description

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

Catalog number	Designation
UP75860A	**MicroBC Assay Protein Quantitation Kit**, sufficient reagents for 500test-tube or 3 400 microplate assays Contains: UP67251A Reagent A, 250mL UP67252A Reagent B, 250mL UP67253A Reagent C, 12mL UP36859A BSA standard, 10x1mL
UP75860C	**MicroBC Assay Protein Quantitation Kit**, sufficient reagents for 50 test-tube or 340 microplate assays Contains: UP67251C Reagent A, 25mL UP67252C Reagent B, 25mL UP67253C Reagent C, 1.2mL UP36859A BSA standard,1mL

Storage: store at room temperature, 1 year from receipt (long term storage at + 4°C)

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

Principle:

The MicroBC Assay is a colorimetric assay: it involves the reduction of Cu2+ to Cu+ by proteins in an alkaline medium. The BC (Bicinchoninic acid) chelates Cu+ ions with very high specificity to form a water soluble purple colored complex. The reaction is increased b high temperatures. As it continues over time, the reaction should be read at a defined time and temperature.

Peptide bonds + Cu2+ ---------- > [tetradente- Cu+ complex]

OH- Cu+ + 2 BC --- > [purple-colored Cu+-BC Assay-Complex]

This reaction is measured by the high optical absorbance of the final Cu+ complex at 562nm. Absorbance is directly proportional to the protein concentration, with a broad linear range between 1µg/mL to 100µg/mL. The protein concentration can be calculated with a reference curve obtained for a standard protein.

Assay Procedure

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

Preparation of samples:

The protein concentration must fall in the range of the standard curve. Therefore it may be useful to prepare several dilutions to meet this requirement: dilute samples if necessary with their respective buffer (alternatively with water).

Each buffer used in samples should be assayed alone to control eventual interference.

Label the tubes and record dilution factor. The dilution factor should be taken into account for the right protein concentration after interpolation from OD562nm. For example:

Sample (name)	Volume sample	Buffer (or water)	Dilution	OD@562 nm	Assayed Protein Concentration (1)	Protein Concentration in Sample (2)
#1: sample1	200	0	1	0.811	67µg/mL	67µg/mL
#2: sample2	20µL	180µL	1/10	0.592	44µg/mL	440µg/mL

(1) Protein concentration = calculated from OD@562nm with the standard curve

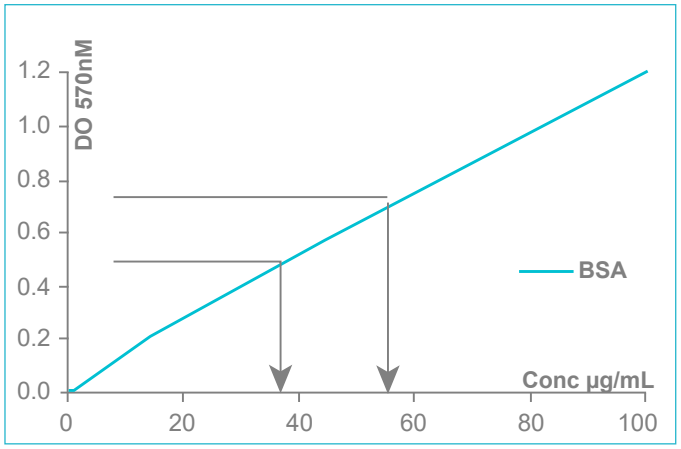
(2) Protein concentration in sample = assayed protein concentration X dilution factor

Preparation of standards:

Uptima recommends to use the protein standard #UP36859A (BSA at 2mg/mL) for most applications.
Prepare a fresh set of protein standards at 100µg/mL to 0.5µg/mL, diluted from the stock solution in the same buffer as the samples (alternatively, water may be used; check the sample buffer by analyzing it versus water).

Standard	BSA standard 2mg/mL #UP36859A	Water or Buffer	Final protein Concentration
Standard A	100µL of Stock	1900µL	100µg/mL
Standard B	666.6µL of A	1000µL	40µg/mL
Standard C	176.5µL of A	1000µL	15µg/mL
Standard D	52.6µL of A	1000µL	5µg/mL
Standard E	25.6µL of A	1000µL	2.5µg/mL
Standard F	10.1µL of A	1000µL	1µg/mL
Standard G	5µL of A	1000µL	0.5µg/mL
Blank H	0	1000µL	0

Figure 1: Typical standard curve with MicroBC Assay #UP75860A



Preparation of the MicroBC Assay reagent (mix A+B+C, 25:25:1):

Prepare the MicroBC Assay reagent by adding 25 parts of reagent A to 25 parts of reagent B, then 1 part of reagent C. Mix well (a temporary turbidity may have appeared).

The table below gives the volume of reagent needed for an assay with the recommended 8 points standard curve:

Test Tube assay (Uniplicates)*

Standard	Number of samples	Reagent A	Reagent B	Reagent C
8 points	1 to 12	10mL	10mL	400µL
	13 to 32	20mL	20mL	800µL
	32 to 52	30mL	30mL	1.2mL

Microplate assay (Duplicates)**

Standard	Number of samples	Reagent A	Reagent B	Reagent C
8 points	8 to 25	5mL	5mL	200µL
	26 to 59	10mL	10mL	400µL
	60 to 94	15mL	15mL	600µL

A duplicate (*), even a triplicate (**) analysis may be recommended for accurate determination; correct volumes accordingly

Use the mixed MicroBC Assay reagent in the next few hours.
Dispose of any unused reagent because possible contamination or degradation may affect further analysis.

Test Tube assay:

	Standard protocol Working range 1µg/mL to 100µg/mL	Enhanced protocol Working range 0.5µg/mL to 40µg/mL
	Allow the reagents to reach room temperature	
a	Pipette 1 ml of each standard, control, and sample into test tubes. Duplicates are recommended.	
b	Add 1 ml of BC Assay reagent (mix A+B+C 25:25:1) per test tube, and mix	
c	Incubate at +37°C for 60min *	Incubate at +60°C for 60 min
d	Cool all test tubes to room temperature and measure the optical absorbance (OD) at 562nm against the blank (water, or buffer + MicroBC Assay reagent.)	
e	Plot the standard curve, and determine the protein concentration.	

Microplate assay:

	Standard protocol Working range 1µg/mL to 100µg/mL	Enhanced protocol Working range 0.5µg/mL to 40µg/mL
	Allow the reagents to reach room temperature	
a	Pipette 150µl of each standard, control, and sample into microplates wells. Duplicates or triplicates are recommended	
b	Add 150µl of BC Assay reagent (mix A+B+C 25:25:1) per test well, and mix (be careful with cross-contaminations)	
c	Incubate at +37°C for 60min *	Incubate at +60°C for 60 min
d	Immediately cool the microplate to room temperature and read the optical absorbance (OD) at 562nm against the blank (water, or buffer + MicroBC Assay reagent). Alternatively, wavelengths from 540 to 590nm have been used.	
e	Plot the standard curve, and determine the protein concentration. An example is given below in figure 1.	

*incubation may be performed for 2-3hours for maximum sensitivity (0.5-5+g/mL)

Scientific Information

Protocol:

Uptima proposes the above standard protocol, that is suitable and convenient for most applications.
Modified protocols may be adopted to suit different custom requirements. However, clients are recommended to take caution when adopting such procedures as this may affect performance i.e.

- increased temperatures (i.e. +60°C) and durations (i.e. 2-3hours) improve the sensitivity. Undesired evaporations may lead to a lower accuracy.
- increasing the volume of sample to reagent can also increase the minimal detected concentration, but take care the assay is not affected because of pH change, or by interfering substances.
- reading the absorbance can be done between 540 and 590nm, for example with microplate readers lacking a 562nm filter. Absorbances, hence the sensitivity, is however not optimal (decreased).

Protein Standard:

Uptima complete kits includes the Bovine Serum Albumin #UP36859A because BSA is a common standard that works for most applications (see below the standard curve). Each user / application may include in the analysis other purified proteins or even any known sample (for example the extract of a reference strain). Ask Uptima for other available standards.

Protein to protein variations:

As with any other protein assay, but with a far lower extent, protein to protein variations may occur to some degree depending on several parameters :

. amino-acid sequences rich in cysteine, tryptophane, tyrosine may increase the MicroBC 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…

The MicroBC Assay can quantitate immobilized proteins (e), for example gel-coupled proteins, or cells adhering to plates.

Interfering / compatible substances:

Some substances may interfere with the BC reaction. It is however remarkable that the MicroBC Assay procedure should be well known for being compatible with a lot of substances, notably most detergents(b). Additionnally, the BC Assay can be combined with protein precipitation kit #R5594A to render it compatible with any small substances, i.e. working for electrophoresis samples (detergent and reducers compatible).
The following table gives some compatible and incompatible substances / concentration:

Compatible (*) Substances	Incompatible Substances
< 3% SDS	Creatinin, Cystein, Tyrosin, Tryptophan
< 2% CHAPS	> 40µM DTT and mercaptans
< 3% Tween 20	Ascorbic acid, H2O2, hydrazides
< 3% Triton X100	EGTA
< 3 M Urea	Phenol Red
< 1% DMSO, DMF	Iron, Copper salts
< 1% Glycerol	Inquire for other substances
< 1 mM PMSF	
< 0.5% NaN3	
TBS (20mM Tris, 150mM NaCl, pH 7.6)	
PBS (0.1M phosphate, 150mM NaCl, pH 7.2)	
Carbonate / Bicarbonate 100mM	
Bicarbonate 40mM	

(*) compatibility is determined if there is less than 10% variation in absorbance for the BSA standard at 40µg/ml. The compatible concentrations may depend on protein nature and concentration.

To limit the interference of some substances[b, c, d]:

- the samples can be diluted provided the protein concentration remains sufficient
- the presence of copper chelators can be overcome by increasing the A:B:C ratio of BC Assay reagent up to 20:30:1 (v/v).
- the interfering substance can be removed (f), for example by prior desalting (dialysis…), precipitation (TCA…), purification… Uptima provides a convenient Protein Precipitation Kit product #R5594A.

In any case, all standards, blanks and controls must be treated in the same way to preserve the accuracy of the assay.

Labware:

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

References:

(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

Other Information

For R&D in vitro use only

For any question, please ask Uptima : uptima@interchim.com ; Hotline: +33 4 70 03 73 06

Catalog size quantities and prices may be found at http://www.interchim.com.