FT-F86400

Coo Protein Assay
Quantitation of proteins by modified Bradford method

Product Description

Reagents for the determination of protein concentration, according to the modified Bradford method (1)
Uptima Coo Protein Assay is the method of choice where high sensitivity, ease of use, or compatibility with reducing substances (sugars, thiols,...) are required.

Catalog number	Designation
UPF86400	**Coo Protein assay**, complete kit, for 500/1000 (tubes), or 4000 (microplate) determinations * Contains: UPF86420 Coo Assay Reagent, 1L UP36859A BSA protein standard, 2mg/mL, 10x1mL
UPF86401	**Coo Protein assay**, complete kit, for 125/250 (tubes), or 1000 (microplate) determinations * Contains: UPF86421 Coo Assay Reagent, 250mL UP36859A BSA protein standard, 2mg/mL, 3x1mL

Storage:	4°C, avoid direct light (L)
Stability:	1 year from purchase date, according to recommended storage conditions

* the number of assays depends on used protocol /concentration assay range and of the volume required for spectrometry measurement

For R&D *in vitro* use only

General Considerations

The determination of protein concentration is required in many applications. Since Bradford introduced a rapid and sensitive colorimetric method (1), this was adopted largely in most labs. The method is based on the binding of a dye (Coomassie) to proteins, changing the color from brown to blue.

Protein + Coomassie® ----------> (Protein: Coomassie®) complex
H+ *blue colour measured at 570-600nm*

Uptima Coo Protein Assay uses this method, providing improved performances:
. It is a **ready-to-use 1-component reagent**, with consistent quality from lot to lot. This avoids the time consuming and pungent preparation of homemade reagent.
. The Coo assay is **very rapid** (few minutes) and **easy** to perform (at room temperature).
. It gives optimal **sensitivity** (up to 1µg of protein/ml) and **reliability** as compared to other formulations: the linearity is optimized to the useful ranges 1-25µg/mL, 20-200µg/mL or 50-1500µg/mL. Your result will be more accurate than with a standard Bradford assay.
. The assay performs well with various samples types: purified proteins, complex mixtures (cell lysates), polypeptides, ...

As a result, Uptima Coo Protein Assay is the colorimetric method of choice in particular when highest sensitivity, ease of use, or compatibility with reducing agents... is required. However on may consider several limitations inherent to coomassie properties (linearity/working range, protein-to-protein variations, interfering substances,...) that can make other procedures preferable, such as methods colorimetric (BC Assay #UP40840A), or fluorimetric (LavaPep #CH4191). See discussion below. Alternatively, CooAssay can be combined with protein precipitation kit #R5594A to render the protein assays compatible with any salt substances. Ask Uptima for any question.

Directions for Use

4 protocols are proposed, depending of requirement of protein assay / samples:

Protocol	Linearity assay range	Ratio vol.sample/reagent
Max Sensitivity protocol	1-25µg/mL	150 + 150
High Sensitivity protocol	20-200µg/mL	25 + 250
Intermediate protocol	50-800µg/mL	10 + 300
Broad Range protocol	50-1500µg/mL	5 + 250

Use only clear recipients (disposable test tubes, microplates, becher…). If recipients should be used again, wash them with a suitable cleaning agent and rinse carefully with distilled water. Traces of proteins or detergents may affect the results.

Preparation of standards, samples and reagents

Mix the Coo reagent bottle (#UPF8642) gently before use and pipette the required amount of Coo reagent. Take care not to splash, or contaminate the reagent bottle when opening and pipetting.

For the maximum sensitivity protocol, prepare first a 25µgml solution (A) with BSA standard stock solution UP36859A. Dilute both standards (see the table below) and samples in the buffer used in the samples (alternatively, water may be used provided a suitable blank is done). Use clear plastic or glass test tubes for sample preparation. It is recommended to make several dilutions to obtain measurements in a same narrow range (i.e. 1-25µg/mL, or 200-800µg/mL) for better accuracy. Include a blank for each buffer.
Do not use a standard vial more than 3-5 times or days because contamination or evaporation may affect the standard.

Assay Protocol performed in Test Tubes (TT)

Use a different set of tubes for incubation of samples/standards with Coo reagent (see the table below for volumes), then read all assays in a glass microcuvette for spectrophotometers. The microcuvette should be rinsed with water between each reading, to prevent the deposit of Coomassie aggregates. Mix the tube and transfer the required volume (usually 1mL) to spectrometer microcuvette before reading.
One can advantageously perform the assay directly in disposable microcuvettes (mix samples+Coo reagent, incubation and reading), provided that the incubations and readings are performed adequately.

Assay Protocol performed in MicroPlates (MP)

Deposit the right volume (see the table) onto microplate wells. After addition of the Coo reagent, mix (30sec with a shaker, or manually, paying attention to cross-well contaminations), then incubate for 1min and read the microplate with a spectrophotometer immediately (within <15min).

Protocol: Linearity range (µg/mL)	**Max Sensitivity** 1-25			**High Sensitivity** 20-200			**Intermediate** 50-800			**Broad Range** 50-1500		
Standards preparation												
starting soln	Prepare standard 'A' (25µg BSA/mL): 20µL of BSA stock std + 1580µL buffer			Use stock Standard solution (2000µg BSA/ml) #UP36859A, provided in the kit UPF8640								
Standards dilutions	**Conc.** µg/mL	**Std** (A)µL	**buffer** µL	**Conc.** µg/mL	**Std** (A)µL	**buffer** µL	**Conc.** µg/mL	**Std** (A)µL	**buffer** µL	**Conc.** µg/mL	**Std** (A)µL	**buffer** µL
	0	0	500	**0**	0	400	**0**	0	400	**0**	0	400
	1	20	480	**20**	5	495	**50**	10	390	**50**	10	390
	2.5	50	450	**50**	10	390	**100**	20	380	**100**	20	380
	5	100	400	**100**	20	380	**200**	40	360	**250**	50	350
	10	200	300	**125**	25	375	**400**	80	320	**500**	100	300
	15	300	200	**150**	30	370	**600**	120	280	**1000**	200	200
	20	400	100	**175**	35	365	**800**	160	240	**1500**	300	100
	25	500	0	**200**	40	360	**1000**	200	200	**2000**	400	0
Assay:	**Microplate**		**Tube**	**Microplate**		**Tube**	**Microplate**		**Tube**	**Microplate**		**Tube**
Volume (µL) of samples, standards, or buffer to pipet	150		1000	25		200	10		50	5		40
Volume (µL) of Coomassie reagent to pipet	150		1000	250		2000	300		1500	250		2000
Mix and **incubate 1mn** at room temperature												
Read ODs at 595nm within 15min against an appropriate negative control (buffer+Coomassie)												

Results

- Plot a **curve** of ODs against the standards dilution

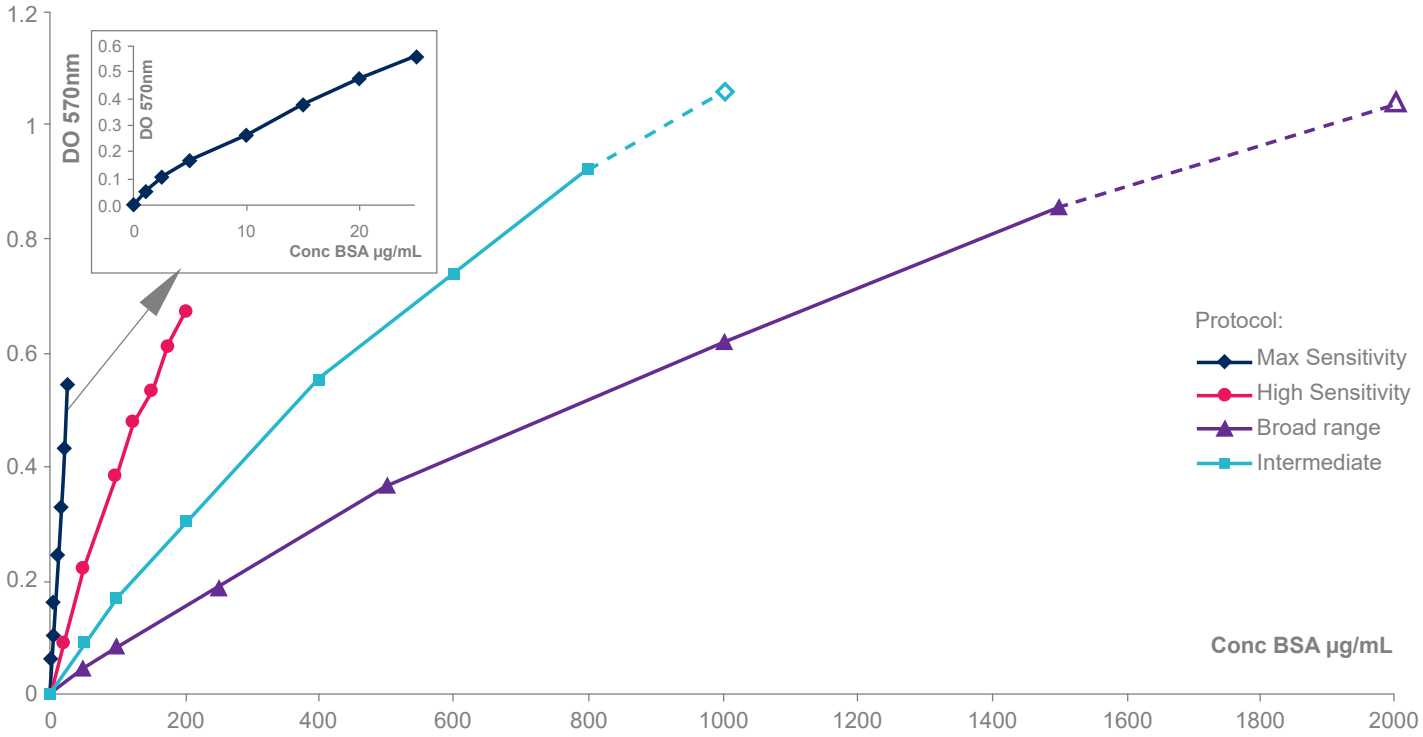

Figure 1: Standard curves of Bovine Albumin Standard (BSA #UP36859), obtained with different protocols used in accordance with the concentration range in which your proteins determination are made.

- **Extrapolate sample proteins concentration** from measured ODs with the standard curve.
This can be performed by hand with a point-to–point drawing, or with a mathematic curve fitting.
Note: linear regression fitting is widely used, but often with insufficient correlation in standard Bradford assays. Uptima reagent and protocols are designed to yield excellent linear regression coefficients, above 0.99 in their respective ranges, as plotted above."

Note: samples with ODs above or below the linear range must be then analysed again more or less diluted.

Scientific and Technical Information

• Coo Assay should be stored properly, at +4°C, and protected from light (it is provided in ambered vials). Improper storage may lead to low absorbencies values.

• The binding of the Coomassie® dye to proteins is very rapid and reliable in the conditions of Uptima procedure. The assay can be performed in flexible conditions (incubation duration, temperature…) without affecting significantly the results. For best reproducibility it is however recommended to proceed always in the same conditions: mix samples / standards with the Coo reagent, then incubate the mixture 1min (<10min), read immediately (within 15min).

. With **incubation** over 30-60min , performances and accuracy are affected by the formation of a precipitate of dye/dye and dye/protein complexes, and by light. Decreasing the temperature may lower slightly optical absorbance. As a result, unexpected low signals may rely on using cold reagent.

. The assay can be read between 570 and 600nm if the 595nm **wavelength** isn't available, with a slight decrease of the sensitivity in comparison with the recommended optimal 595nm measurement.

. For some applications, the **volume ratio** sample / Coo reagent can be modified. The sensitivity can be increased with sample/reagent volume ratio up 4 / 1, but loss of other performances should be appreciated (decrease in linearity, standard working range, and compatibilities).

• The Coomassie® dye binds to primarily basic and aromatic amino-acid residues. This property leads to **protein-to- protein variations of signal**, especially with proteins or peptides rich in arginine, phenylanaline and tyrosine. Also, signal variations are related to amino acid sequence, isoelectric point, structure and the presence of certain side chains or prosthetic groups. Lastly, protein-to-protein variations may vary depending on interfering substances. I.e. surfactant may lead to protein/dye precipitates.

-The resulting bias between samples and the (BSA) standard can be avoided using a purified protein similar to samples.

-Bias between different protein types can be reduced choosing an other method with a lower protein-to-protein variation such as the BC Assay #UP40840. Please ask Uptima.

-**Peptides** (<5000MW) or even polypeptides may show low absorbencies values, prompting to choose a more appropriate assay, such as LavaPep #CH4191.

• The complete kit includes a common **standard**, bovine albumin that has a high detection signal. This is suitable for most applications. For more accurate results, use the same protein than the one analyzed in the samples, for example the purified studied protein, or a similar reference protein mixture.

• Some substances that are present in the buffer, during a purification step, or in proteous extracts from cells, may interfere with the color response.

Compatibility was shown for many compounds, including some detergents (CHAPS, CHAPSO, Octyl-b-thioglucopyranoside at 2.5%+ ; NP-40, DOC at 0.3%+), chaotropic agents (Guanidine, Urea at 3M+), reducers (KSCN , b-ME, DTT at 5mM+), most solvents (Acetone, acetonitrile, ethanol, methanol at 10%), acids and bases (HCl, NaOH at 100mM), most buffers at 100mM+ (carbonate, phosphate, citrate, borate, but also Good's buffers i.e. HEPES, MOPS, Tris 1M+), some salts (Ammonium Sulfate, Tris at 1M), chelating agents (EDTA 100mM), Sucrose, Glycerol..

Interferences/low compatibilities are known or should be checked with many detergents (SDS, Brij and Tween), several salts and biochemicals (amino-acids (asparagin), lipids, preservatives (sodium azide, thimerosal at 0.1%+), several salts (chlorides of Co Fe En), strong alkali compounds (NaOH 100mM), Glucose (1mM) See list of tested compounds and compatible concentrations.

• To minimize / eliminate the effects of interfering substances there are several options:
- **Dilute sample** to the point of no interference (useful if limited interference, and sufficient protein concentration)
- Remove the interfering substance by dialysis or other suitable desalting method.
- Precipitate out the proteins with acetone or trichloroacetic acid. After the contaminated liquid containing the 'interfering substance' being disposed off, the protein pellet can then be solubilized in a small volume of water / CooAssay reagent. Uptima provides a convenient Protein Preparation product **#R5594A** - room temperature!.
- Choose an other assay method (ask Uptima for the BC Assay or LavaPep.).

Other Information

For any information, please contact Uptima at: uptima@interchim.com ; Hotline: +33 4 70 03 73 06
Catalog size quantities and prices may be found at http://www.interchim.com.

Related products and documents

Comparison of Coo Assay with competitors: NT-UPF8640a
Other products using BioSciences Innovations catalogue and e-search tool:
Protein Preparation product **#R5594A** ;
Protein TCA precipitation reagent #BI2941 other protein assays (BC Assay **#UP40840**, LavaPep **#CH4191**)
desalting tools (CelluSep, Desalting columns **#UP848742**)

Literature

Bradford M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal.Biochem., 72, 248-254 (1976)

Sedmak, J.J. and Grossberg, S.E. (1977). A rapid and sensitive versatile assay for protein using Coomassie® brillant blue, Anal.. Biochem. 79, 544-552