# UPF8640 Coo Protein Assay

## Quantitation of proteins by the 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 | Coomassie Reagent, 1 L |
| | | UP36859A | BSA protein standard, 2mg/ml, 10x1ml |
| UPF86401 | Coo Protein assay, complete kit, for 125/250 (tubes) , or 1000 (microplate) determinations * | | |
| | Contains | UPF86421 | Coomassie Reagent, 250ml |
| | | UP36859A | BSA protein standard, 2mg/ml, 3x1ml |

Storage : 4°C, avoid direct light (b)

Stability: 1 year from purchase date, according to recommended storage conditions

For R&D *in vitro* use only

* the number of assays depends on used protocole and of the volume required for spectrometry measurment

## 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, known as Coomassie, for proteins, followed by a colour modification from brown to blue.

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

Uptima formulated a ready-to-use 1 component reagent to give optimal results, in terms of sensitivity (upto 1µg of protein/ml), and reliability as compared to other formulations: the method is very quick and easy to perform, the linearity is optimized to the useful ranges 1-25µg/ml, 20-200µg/ml or 50-1500µg/ml.

The assay was performed successfully to various samples: purified proteins, complex mixtures (cell lysates), polypeptides, immobilized proteins… But some characteristics of the method (linearity, protein to protein variations, interfering substances) must be considered in each application (see the review below).

Uptima Coo Protein Assay is the method of choice where highest sensitivity, ease of use, or compatibility with reducing agents… are required. An other procedure should be considered in applications where compatibility is required for other agents, notably detergents, high ionic strengh. We then recommend the BC Assay kit (#UP40840A). Ask Uptima for any question.

# Directions for Use

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

- **-Broad Range protocol** : assay linear from 50 to 1500µg/ml (ratio 5 vol. sample + 250 vol. reagent)
- **-Intermediate protocol**: assay linear from 50 à 800 µg/ml (ratio 10 vol. sample + 300 vol. reagent)
- **-High sensitivity protocol**: assay linear from 20 à 200 µg/ml (ratio 25 vol. sample + 250 vol. reagent)
- **-Max sensitivity protocol** : assay linear from 1 à 25 µg/ml (ratio 150 vol. sample + 150 vol. reagent)

Use only clear recipients (disposable test tubes, microplates, becher…). If recipients should be usedagain, wash them with a suitable cleaning agent and rince 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 protocole, prepare first a 25µgml solution (A) of BSA standard with 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 toobtain measurements in a samenarrow 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 further results.

Assay Protocol performed in Test Tubes (TT)

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

Assay Protocol performed in Micro Plates (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) andincubate fro 1min and read the microplate with a spectrophotometer immediately (or within 15min).

| *Protocols* | **Max Sensitivity** | **High Sensitivity** | **Intermediate** | **Broad Range** |
|---|---|---|---|---|
| *linearity range (µg/ml)* | **1-25** | **20-200** | **50-800** | **50-1500** |
| *Standards dilutions* | solution A.: 25µg/ml<br>20 µl sol stock +<br>1580 µl buffer | stock Sol.: 2000µg/ml<br>(UP36859A)<br>* provided in the kit UPF8640 | stock Sol.: 2000µg/ml<br>(UP36859A)<br>* provided in the kit UPF8640 | stock Sol.: 2000µg/ml<br>(UP36859A)<br>* provided in the kit UPF8640 |
| | **Conc. µg/ml** / **Sol A µl** / **buffer µl**<br>**0** 0 500<br>**1** 20 480<br>**2.5** 50 450<br>**5** 100 400<br>**10** 200 300<br>**15** 300 200<br>**20** 400 100<br>**25** 500 0 | **Conc. µg/ml** / **Stock Sol µl** / **buffer µl**<br>**0** 0 400<br>**20** 5 495<br>**50** 10 390<br>**100** 20 380<br>**125** 25 375<br>**150** 30 370<br>**175** 35 365<br>**200** 40 360 | **Conc. µg/ml** / **Stock Sol µ** / **buffer µl**<br>**0** 0 400<br>**50** 10 390<br>**100** 20 380<br>**200** 40 360<br>**400** 80 320<br>**600** 120 280<br>**800** 160 240<br>**1000** 200 200 | **Conc. µg/ml** / **Stock Sol µl** / **buffer µl**<br>**0** 0 400<br>**50** 10 390<br>**100** 20 380<br>**250** 50 350<br>**500** 100 300<br>**1000** 200 200<br>**1500** 300 100<br>**2000** 400 0 |
| *Volume (µl) of samples, standards, or buffer to pipet* | Microplate **150** / Tube **1000** | Microplate **25** / Tube **200** | Microplate **10** / Tube **50** | Microplate **5** / Tube **40** |
| *Volume (µl) of Coomassie reagent to pipet* | **150** / **1000** | **250** / **2000** | **300** / **1500** | **250** / **2000** |
| Mix and incubate 1 mn at room temperature | | | | |
| Read ODs at 595 nm withn 15min against an appropriate negative control (buffer+Coomassie) | | | | |

Results

-Plot a curve of ODs against the standards dilution

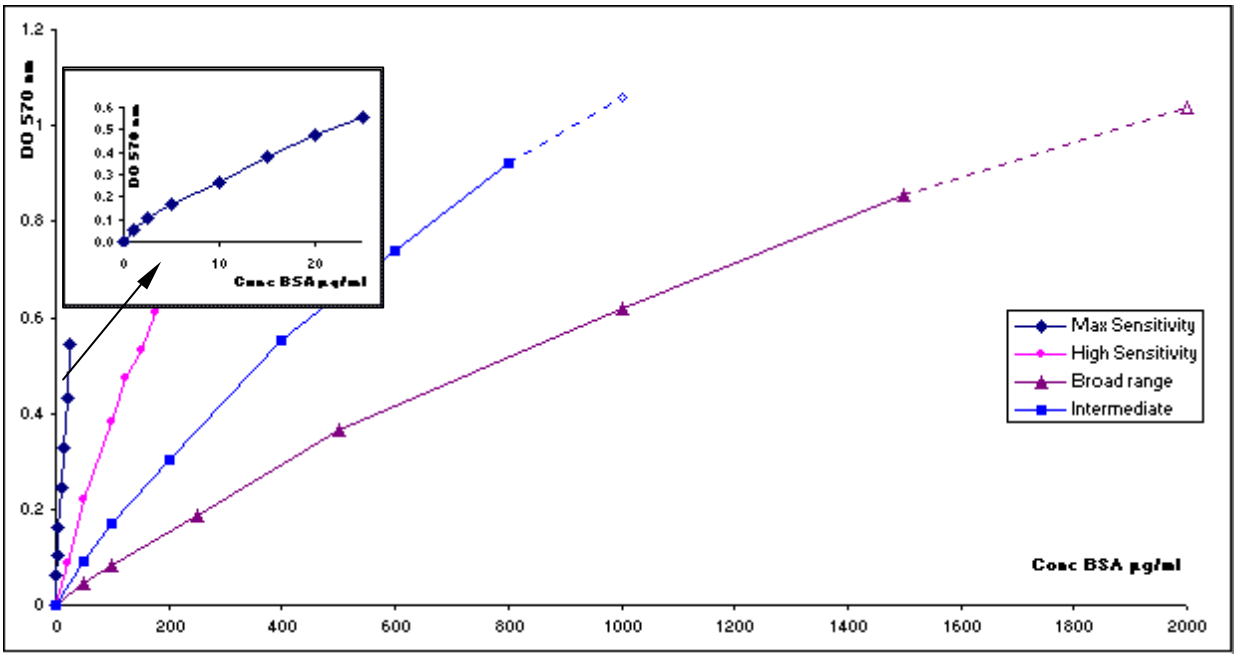


Figure 1 : Standard curves of Bovine Albumine Standard (BSA #UP36859), obtained with differents protocols used in accordance with the concentration range in which your proteins determination are made.

-Extrapolate sample proteins concentration from measured ODs with the standard curve.

This can be perfomed by hand with a point-to –point drawing, or with a mathematic curve fitting *

*: take care to check for right correlation.

A quadratic fit is preferable. Linear regression fitting is widely used, although not suitable for commonly working ranges. The linear regression coefficient must be above 0.99. Uptima protocols are adapted to following linear ranges:

| *Protocol* | *linearity range* |
|---|---|
| Max Sensitivity protocol | 1-25µg/ml |
| High Sensitivity protocol | 20-200µg/ml |
| Intermediate protocol | 50-800µg/ml |
| Broad Range protocol | 50-1500µg/ml |

-The ODs above or below the linear range must be then analysed again more or less diluted.

# Scientific and Technical Information

- The binding of the Coomassie® dye to proteins is very rapid and reliable in the conditions of Uptima procedure. The assay can be perfomed in flexible conditions (incubation duration, temperature …) without affecting significantly the results. However, 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 (up10min), read immediately (or within 15min).
  .Coo Assay should be **stored** properly, at +4°C, and protected from light (it is provided in ambered vials ). Unproper storage may lead to low absorbances values.
  .Over 30-60min **incubation**, performences 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 a little optical absorbance. (d) 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 care fully, check for a related decrease in linearity, standard working range, and compatibilities.

- The Coomassie® dye binds to primarily basic and aromatic amino-acid residues. This property lead to **protein-to-protein variations of signal**, especially with proteins or peptides rich in arginine, phenylanaline and tyrosine (c). 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. Uptima Coo Assay elicits slightly reduced protein-to-protein variations. If a very low protein-to-protein variation is necessary, either choose the right protein standard for each sample, or choose an other method (ask Uptima for the BC Assay #UP40840).
- **Peptides** (<5000MW) or even polypeptides may show low absorbances values. Choose a more appropriate assay, BC Assay #UP40840
- The complete kit includes a common **standard**, bovine albumin, that shows a high detection signal. This is suitable for most applications. For more accurate results, use the same protein than the one analysed in the samples, for example the purified studied protein, or a similar reference protein mixture. For 3-4 fold lower protein-to-protein variations, use the BC Assay #UP40840.
- Some substances that are present in the buffer, during a purification step, or in proteic extracts from cells, may interfere with the colour response. **Compatibility** was shown for many compounds, including 0.125% TritonX100, 80mM DTT, 0.1M Bicarbonate, Chaps 2.5%, Tris 2M. However, **Incompatibilities** are well known with detergents (excepted CHAPS, CHAPSO, Octyl-b-thioglucopyranoside, Urea, TritonX100), basic amino-acids (asparagin), lipids, sodium azide (at 0.5%)… Alkali compounds (or in too much high volume of sample / reagent) lead to high absorbances even without proteins.. Measurement in presence of these substances can be done after dilution (useful if limited interference, and sufficent protein concentration), after removal by dialysis or other suitable dessalting method, or by the TCA precipiation (ask Uptima), or with an other assay method (ask Uptima for the BC Assay #UP40840).

# Other Information

For any question, please ask Uptima

Registered trademarks:
From ICI Americas: Coomassie® and Tween®

NT-UPF8640(comparison)

**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

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