FT-36858A

CooAssay Standard Protein Assay Kit

Quantitation of proteins by a modified Bradford method

Product Description

Catalog number	Designation		
UP36858A	**CooAssay Standard Protein Assay Kit**		
	for 500/1000 (tubes), or 4000 (microplate) determinations *		
	Contains	UP36858a	CooAssay Reagent, 1 L
		UP36859A	BSA protein standard, 2mg/ml, 10x1ml
UP36858C	**CooAssay Standard Protein Assay Kit**		
	for 125/250 (tubes) , or 1000 (microplate) determinations *		
	Contains	UP36858b	CooAssay Reagent, 250ml x2
		UP36859A	BSA protein standard, 2mg/ml, 5x1ml

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

Storage : 4°C, avoid direct light (L)

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

The Uptima CooAssay is a high sensitivity total Protein Assay Kit compatible with reducing substances. Protein-to-Coomassie binding facilitates a brown to blue change in color and establishes absorption of 595 nm. Microplate and test tube protocols are provided that rely on mixing and incubating a small amount of sample containing protein with the CooAssay reagent, followed by measurement at 595 nm. A standard curve is constructed using standard protein dilutions (typically BSA) forming a reference for the unknown samples. It is essential to perform a standard curve for each assay.

Directions for Use

Preparation of BSA Standards (Diluted) for Standard Curve

Use only clear recipients, preferably disposable items, as traces of proteins or detergents may affect the results.

For protocols with a **working range = 100-1 500µg/ml**

Vial	Diluent Volume (µl)	BSA (µl)	Final BSA Conc.(µg/ml
1	0	100µl from BSA 2mg/ml vial	2000
2	100µl	300µl from BSA 2mg/ml vial	1500
3	200µl	200µl from BSA 2mg/ml vial	1000
4	250µl	150µl from BSA 2mg/ml vial	750
5	300µl	100µl from BSA 2mg/ml vial	500
6	350µl	50µl from BSA 2mg/ml vial	250
7	375µl	25µl from BSA 2mg/ml vial	125
8	395µl	5µl from BSA 2mg/ml vial	25
9	400µl	0	0 (blank)

For protocols with a **working range = 1-25µg/ml**

Vial	Diluent Volume(µl)	BSA (µl)	Final BSA Conc.(µg/ml)
1	12640µl	160µl from BSA 2mg/ml vial	25
2	800µl	3200µl from vial 1	20
3	1600µl	2400µl from vial 1	15
4	2400µl	1600µl from vial 1	10
5	3200µl	800µl from vial 1	5
6	3600µl	400µl from vial 1	2.5
7	3840µl	160µl from vial 1	1
8	4000µl	0	0 (blank)

Preparation of BSA Standards (Diluted) for Standard Curve

The table on page one outlines a recommended set of protein concentrations. Use one BSA provided vial and the same diluent as the samples (there is sufficient volume for 3 duplications of each dilution).

Note: Do not use a standard vial more than 3-5 times or days because contamination or evaporation may affect the standard. For accurate results, refer below to 'Further Information/Standard'.

Preparation of samples

Dilute samples preferably in the sample buffer or alternatively, water may be used provided a suitable blank is performed. It is recommended to make several dilutions to obtain measurements in a same narrow range for better accuracy. Include a blank for each buffer if necessary.

Preparation of CooAssay Reagent

The CooAssay Reagent should be stored properly, at +4°C, and protected from light (it is provided in an amber bottle). Improper storage may lead to low absorbance values.

Coomassie reagents frequently form dye-dye aggregates on standing and it is common for such aggregates to be visible as a dark precipitate. Gentle mixing elevates this problem and this should be performed immediately prior to use by a gentle turning of the bottle several times. The bottle should not be shaken to mix the solution.

For accurate protein assays it is essential to mix the CooAssay reagent before each use and also to mix each tube/plate immediately prior to measuring the absorbance. Only remove the amount of reagent required and allow the solution to reach room temperature before use. Take care not to splash, or contaminate the reagent bottle when opening and pipetting.

A1: Assay Protocol performed in MicroPlates (MP) - Working Range = 100-1 500 µg/ml

1. Pipette 5 µl of standard/ unknown sample into microplate wells.
2. Add 250 µl of the CooAssay dye to each well and plate mix (shaker) for half a minute*.
3. Incubate plate for 10 minutes at room temperature.
4. Use a plate reader to measure absorbance at 595nm.
5. Measure OD values for all individual standards / unknown samples. Subtract the average Blank reading from each.
6. Construct a standard curve. The curve is established by plotting the net absorbance at 595 nm measurements for each BSA standard vs. concentration in µg/ml **.
7. Determine unknown sample protein concentration using the standard curve.

A2: Assay Protocol performed in MicroPlates (MP) - Working Range = 1-25 µg/ml

1. Pipette 150 µl of standard/ unknown sample into microplate wells.
2. Add 150 µl of the CooAssay dye to each well and plate mix (shaker) for half a minute*.
3. Incubate plate for 10 minutes at room temperature.
4. Use a plate reader to measure absorbance at 595nm.
5. Measure OD values for all individual standards / unknown samples. Subtract the average Blank reading from each.
6. Construct a standard curve. The curve is established by plotting the net absorbance at 595 nm measurements for each BSA standard vs. concentration in µg/ml **.
7. Determine unknown sample protein concentration using the standard curve.

B1: Assay Protocol performed in Test Tubes (TT) - Working Range = 100-1 500 µg/ml

1. Label test tubes. Add 30 µl of each standard/ unknown sample.
2. Add 1.5 ml of the CooAssay dye and mix. Incubate for 10 minutes *.
3. Measure the absorbance of all standards and samples with a spectrophotometer to 595nm (zeroed with a cuvette filled with water, or subtract values with a Blank).
4. Construct a standard curve. The curve is established by plotting the net absorbance at 595 nm measurements for each BSA standard vs. concentration in µg/ml **.
5. Determine unknown sample protein concentration using the standard curve.

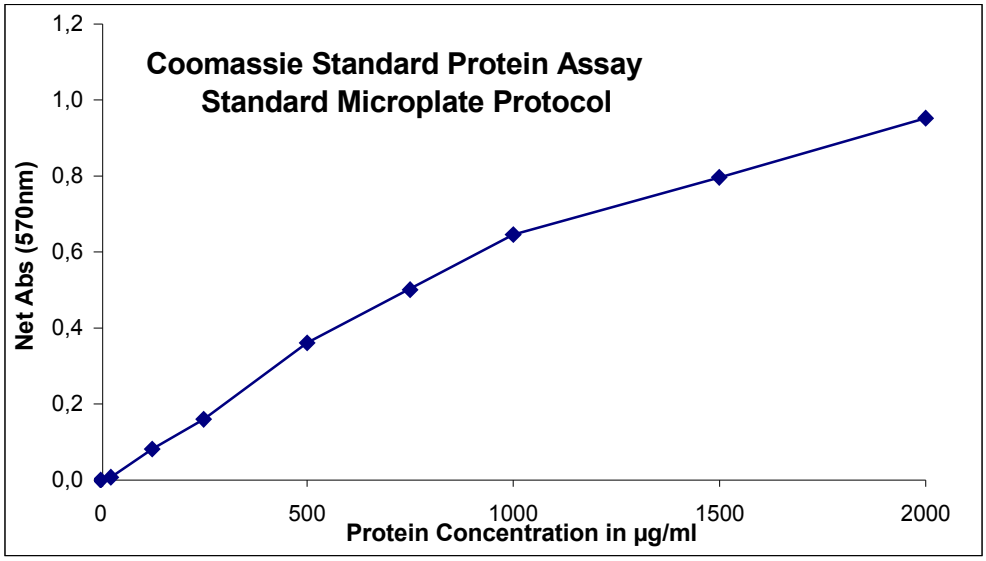
B2: Assay Protocol performed in Test Tubes (TT) - Working Range = 1-25 µg/ml

1. Label test tubes. Add 1.0 ml of each standard/ unknown sample.
2. Add 1.0 ml of the CooAssay dye and mix. Incubate for 10 minutes *.
3. Measure the absorbance of all standards and samples with a spectrophotometer to 595nm (zeroed with a cuvette filled with water, or subtract values with a Blank).
4. Construct a standard curve. The curve is established by plotting the net absorbance at 595 nm measurements for each BSA standard vs. concentration in µg/ml **.
5. Determine unknown sample protein concentration using the standard curve..

* Internal studies have shown that a 10 minutes room temperature incubation improve consistency and signal over quick reading (i.e.1min).
** Plotting results: A point-to-point curve is preferable to a linear fit to the standard points. If using a curve-fitting algorithm from the microplate reader avoid a linear fit. i.e. select a quadratic regression or best-fit curve.

Results

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.



Additional Information

Interfering / compatible Substances

Many ionic & non-ionic detergents are known to interfere with Coomassie. In such instances color development is inhibited & precipitation may occur. Certain interfering substances may be tolerated at reduced concentrations.

Known interference is shown in the presence of:

SDS, Brij and Tween,
A number of salts (chlorides of Co Fe En),
Biochemicals (amino-acids, asparagin),
Lipids,
Preservatives (sodium azide, thimerosal at +0.1%),
Strong alkali compounds (NaOH 100mM), Glucose (1mM).

To minimize / eliminate the effects of interfering substances there are several options:

- Dilute sample to the point of no interference (useful if limited interference, sufficient protein concentration)
- Remove the interfering substance by dialysis or another suitable desalting method
- Precipitate out the proteins.
  Adding acetone or trichloroacetic acid TCA, and centrifugation can achieve this. Dispose the supernatent containing the 'interfering substance' and solubilize the protein pellet with water / CooAssay reagent.

Compatibility is known for many compounds i.e

Certain 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),
Additives such as Chelating agents (EDTA 100mM), Sucrose, Glycerol..

Further Information

Glassware : Attempt to use disposable polystyrene cuvettes, the Coomassie dye will stain glass or quartz cuvettes.

Cleaning: All glassware must be thoroughly cleaned, if a detergent is used make sure that it is completely removed in the final rinse. Traces of proteins or detergents may affect results.
Absorbance Reading: The blue color may be measured within a wavelength range typically from 570 nm and 610 nm but this will result in a lower slope for the standard curve and may lower the detection sensitivity for the protocol. Maximum assay sensitivity is shown at 595nm absorbance

Temperature: The CooAssay reagent should be stored at 4ºC but allowed to reach room temperature for the whole assay 595 nm protocol measurements are optimized with working reagent established at room temperature

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

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

Other Information

For R&D *in vitro* use only

Trademarks: Coomassie® and Tween® from ICI Americas; CooAssay from Interchim

For any question, please ask Uptima

Contact your local distributor

Uptima, powered by
interchim
213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

Uptima@interchim.com