

The study of proteins structure gained peculiar importance with the development of proteomics. After the course to decrypt nucleic coding sequences, the research community started to decrypt extensively protein sequences. Hot issues regards new developments in proteins structure and protein expression, as well quantitatively than regarding localization in cells and qualitatively (post-translational modifications). Interchim is proud to present here a selection of useful tools for proteomics, including standard analysis (**Protein assays, Electrophoresis**) and more specific tools for proteomics, **micro arraying** : protein expression systems for in-vivo expression study (**reporter assays**) and **recombinant protein** production, protein **expression silencing**, and **molecular crystallography** for protein structure elucidation.

Protein Assays

The proteins determination in solution is performed by various methods. Among these, colorimetric methods are widely used, because of interesting features especially conveniency, sensitivity. Most of them suit polypeptides, but only a few work properly for small peptides.

Protein assays – Colorimetric assays

How to choose the right Protein Assay?

Every protein assay exhibits restrictions of use depending on applications. The most useful features to consider are :

- ◆ Sensitivity (lower detection level)
- ◆ Signal variation from protein to protein
- ◆ Compatibility with substances found in samples
- ◆ Signal linearity with protein concentration .

Uptima offers 3 colorimetric assays that cover most applications. These high quality and optimized formulated reagents overcome most of conventional methods, whilst fulfilling most requirements of sensitivity, compatibility, accuracy and ease-of-use. Select the most important features and find in the following table the assay that answers the best to your requirements, in regard of performance.

Selection guide

Measure of absorbance	Generally based on the optical absorbance of peptidic bounds (215 nm) or aromatic amino-acids (280 nm). Very simple and rapid. The user should know the exact extinction coefficient of the assayed protein(s). Furthermore, this methods is often leant over by, or does not suit samples with numerous compounds in buffer (Salts, DMSO, detergents...), nor UV absorbing contaminants (for example hemoglobin).
Kjendal method	Colorimetric assay; very low sensitivity.
Biuret method	Colorimetric assay, based on the reduction of Cu ⁺⁺ by peptidic bond in alkaline conditions; low sensitivity.
Lowry method	Improved Biuret assay (the folin-Ciocalteu reagent increases the color development); reading at 750 nm ; not very convenient (prepare freshly reagents each day; 2 incubations, timed operating and temperature control); noticeable interferences with compounds found in biological samples.
Bicinchoninic method	Colorimetric assay, based on the chelating of Cu ⁺⁺ ions by bicinchoninic acid producing an intense purple color; very linear and wide standard curve.
Bradford method	Colorimetric assay based on the interaction of a dye (Coomassie) with some amino-acids ; well known and used, but a lot of modified procedures. Important protein-to-protein signal variations.
Fluorimetric methods (OPA method)	Fluorimetric high sensitive method (down 50ng/ml). Suits also for small peptides (dependent sensitivity on lysine content, because based on the reaction with amines from lysine residues).
(others)	Several other reagents similar to OPA are use to derivatize aa, peptides and proteins before analysis (Chromatography, Electrophoresis,...). Some are useful for assaying proteins, glycoproteins or other biomolecules. Our ProRed(FP503) allows protein assay in solution and gels.

Product	Suits especially to applications where are needed	Drawbacks
BC Assay UP40840	Accuracy, versatility, compatibility*, along with good sensitivity (*notably with detergents, bases, nucleic acids, lipids...)	Incompatible with reducing agents, some chelators
MicroBC Assay UP75860	As BC Assay, but 4-6 fold more sensitive !	Same as BC Assay
Coo Assay UPF8640	Quick of use, and compatibility with reducing agents	Incompatible with most detergents, lipids, alkalis... Protein-to-protein variations

Technical tip

Proteomics

The term "proteome" arise in 1995 to introduce the concept of "protein map" (Wasinger et al). used a combination of two-dimensional gel electrophoresis, amino acid composition analysis, MALDI-TOF mass spectrometry, and N-terminal Edman degradation to analyze the protein complement, or proteome of the organism *Mycoplasma genitalium*. This combination of technologies allowed proteins to be identified prior to detection of their respective genes. Now, proteomics deals not only the protein identity and diversity in a sample, but also with their respective abundance, dynamics, and modifications.

Biochemistry and molecular level analysis are essential to proteomics knowledge development.



Biochemistry & Molecular Biology Analysis

Protein Assays

	BC Assay UP40840A (modified lowry assay, based on bicinchoninic acid) <i>A very versatile and efficient assay, suitable for most applications. Detergents compatible.</i>			MicroBC Assay UP75860 (modified lowry assay, based on bicinchoninic acid) <i>The high sensitivity version of the BC Assay</i>	Coo Assay (Bradford) UPF86400 (modified Bradford assay, based on Coomassie dye) <i>A very popular assay, worldwide known and documented. Reducing agent compatible.</i>			
Protocol	Standard Assay	Room Temperature	Enhanced	Standard Assay	Broad range	Intermediate	High Sensitivity	Max Sensitivity
Reagent prep.	Mix 2 components A+B (50:1)			mix 3 components A+B+C (25:25:1)	1 ready-to-use reagent (Coo)		1 ready-to-use reagent (Coo)	
Sample (in tube microplate)	100µl (25µl)	100µl (25µl)	100µl (25µl)	1ml (150µl)	40µl (5µl)	50µl (10µl)	200µl (25µl)	1ml (150µl)
Reagent	2 ml (200µl)	2 ml (200µl)	2 ml (200µl)	1ml (150µl)	2ml (250µl)	1.5ml (300µl)	2ml (250µl)	1ml (150µl)
Incubation	30min at 37°C	2h at Room Temperature	30mn at 60°C	1h at 37°C	1min at Room Temperature			
OD reading	562nm (540-590nm)			562nm (540-590nm)	595nm (570-610nm)			
Comments	Flexible protocols (temperature and duration) (1 minute protocol with microwave heating) Great accuracy				Very easy and quick No filtration needed!			

Performances / features

Accuracy, Linearity	Very good, 20-1000µg/ml Overcomes often Coomassie assay			Very good, 1-100µg/ml	50-1500µg/ml	50-800µg/ml	20-200µg/ml	1-25µg/ml
Protein to protein variations	Low (3-4 fold lower than with the Coo Assay)			Low (slightly better than BC Assay)	Noticeable	Noticeable		
Sensitivity and working range	20-2000µg/ml	5-500µg/ml		1-200µg/ml	50-2000µg/ml	50-1000µg/ml	20-500µg/ml	1-100µg/ml
Stability	>1 year at Room Temperature			>1 year at Room Temperature	1 year at 4°C			
Compatibility	Most detergents, chaotropic agents, preservatives (anti-microbials...), inhibitors (of proteases...), buffer and salts			Similar to BC Assay, but compatible concentrations are slightly lower because of sample/reagent volume ratio	Reducing agents (80mM DTT...), 2% Triton X100, 400mM Bicarbonate...			
Incompatibility	Reducing agents (sugars, thiols...), copper chelators, cys, tyr, try amino-acids			Same as for the BC Assay	Incompatible with numerous detergents, strong Alkaline solutions			

Applications

Samples	Purified proteins, complex mixtures, polypeptides, immobilized proteins... Notably containing : nucleic acids, lipids, detergents, alkalis ...	Purified proteins, complex mixtures, polypeptides... Notably containing: nucleic acids, lipids, detergents, alkalis ...	Purified proteins, complex mixtures Acidic samples Reducing agents containing samples
Others	Determination of Copper concentration		

Cat.# Qty
UP40840A BC Assay
protein Quantitation Kit, complete kit
 for 500 (tube) or 5000 (microplate) determinations
 Contains* : UP95424A, reagent A, 1 L
 UP95425A, reagent B, 25 ml
 UP36859A, Serum Bovine Albumin Standard, 2 mg/ml, 10x1ml

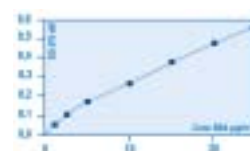
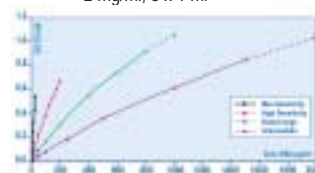
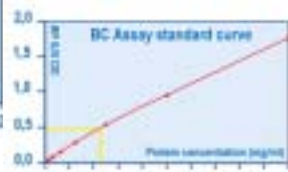
UP40840B BC Assay
protein Quantitation Kit, complete small kit
 for 125 (tube) or 1250 (microplate) determinations
 Contains* : UP95424B, reagent A, 250 ml
 UP95425B, reagent B, 6 ml
 UP36859A, Serum Bovine Albumin Standard, 2 mg/ml, 3x1ml

Cat.# Qty
UP75860A Micro BC Assay
protein Quantitation Kit, complete kit
 for 500 (tube) or 3400 (microplate) det.
 Contains* : UP67251A, reagent A, 250 ml
 UP67252A, reagent B, 250 ml
 UP67253A, reagent C, 12 ml
 UP36859A, BSA standard, 10x1ml

UP75860C Micro BC Assay
protein Quantitation Kit, complete small kit
 for 50 (tube) or 340 (microplate) det.
 Contains* : UP67251C, reagent A, 25 ml
 UP67252C, reagent B, 25 ml
 UP67253C, reagent C, 1.2 ml
 UP36859A, BSA standard, 1x1ml

Cat.# Qty
UPF86400 Coomassie Protein Assay,
complete classic kit
 for 500 (tube) or 4000 (microplate) determinations
 Contains* : UPF86420, Coomassie reagent, 1 L
 UP36859A, Bovine albumin standard, 2 mg/ml, 10 x 1 ml

UPF86401 Coomassie Protein Assay,
complete small kit
 for 125 (tube) or 1000 (microplate) determinations
 Contains* : UPF86421, Coomassie reagent, 250 ml
 UP36859A, Bovine albumin standard, 2 mg/ml, 3 x 1 ml



*All components of kits available separately

Protein assays - Fluorogenic

OPA Protein Quantitation Kit

This kit is designed to quantify proteins as well as peptides (even small ones) in solution in the range from 50 ng/ml to 25 µg/ml. It uses OPA reagent for rapid and sensitive protein detection. Unlike home-made protocols and other kits, that require a thiol compound (such a 2-mercaptoethanol); it does contain odorless chemicals thanks to a proprietary formulation. The kit works well in presence of lipids, reducing agents and detergents. OPA assay kit has been shown to give faster and more sensitive detection of peptides and proteins than most other conventional methods, including all colorimetric methods. But it do not suit to acetylated and other primary amine-blocked peptides.

Description	Cat.#	Qty
OPA Protein Quantitation Kit	51225A	500 tests

See descriptions :

ABD-F	FP-57564A	100 mg	page B91
ANTS	FP-46574A	500 mg	page B.83
APTS	Fp-33972A	10 mg	page B84
DMEQ	FP-69129A	10 mg	page B84
SBF-CI	FP-AM859A	10 mg	page B82

Derivatization reagents for AA, Peptides and Proteins

Most of labeling fluorochromes described pages B51-B84, can be used to derivatize small molecules for bioanalysis as chromatography and capillary electrophoresis. Here is selected interesting or popular fluorochromes.

- ◆ Detection of thiols : CPM, OPA, ABD-F
- ◆ Detections of amine : OPA, ProRedFP503, SBF-CI
- ◆ Detection of alcohols : DMEQ
- ◆ Detections of aldehydes & ketones : APTS, ANTS

OPA

$\lambda_{abs}/\lambda_{em}$: 330-390 nm / 436 nm
MW : 134.1

Amine reactive fluorogenic agent. It is included in our Protein assay 51225A, that avoid to use pungent thiols required in the reaction. Can also be used to detect thiols.

Description	Cat.#	Qty
OPA (o-phthalaldehyde)	FP-02727F	1 g

ProRedFP503

$\lambda_{abs}/\lambda_{em}$ (free) : 503/565 nm
 $\lambda_{abs}/\lambda_{em}$ (coupled) : 540/600 nm

A new dye that is quite non-fluorescent until it reacts with protein to give a fluorescent compound detected with superior sensitivity and selectivity. Used for peptide and protein quantitation in solutions, and also for proteins in electrophoresis gels (protocol on inquire).

Description	Cat.#	Qty
ProRedFP503	FP-BC2011	1 mg

Ninhydrin

$C_9H_6O_4 \cdot H_2O$
MW : 178.1

Reacts with aldehydes and primary amines to form highly fluorescent ternary compounds. Also used as colorimetric indicator of aminoacids and primary aliphatic amines in biochemistry analysis (TLC).

Description	Cat.#	Qty
Ninhydrin monohydrate	024400	25 g
	024404	100 g
	228832	10 ml (10% son in ethanol)

