

# BC ASSAY PROTEIN ASSAY REAGENT

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Reliable, sensitive and specific reagents  
for protein quantification

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**Advion Interchim**  
scientific

# DID YOU KNOW...

Interchim® and the BC Assay have a long history of more than 25 years!  
Our BC Assay is manufactured in France, in our ISO9001 certified laboratory.  
Ordering our BC Assay provides many benefits, including:

- The quality of manufacturing that we bring to all our reagents including the BC Assay.
- Advion Interchim Scientific technical support on protein quantification.
- Competitive rates: We are producers and resellers, with no intermediary between you and us.
- Fast delivery time. We maintain a stock on the BC Assay. Delivery is 24-48 hours after receipt of your order.

BC Assay protein quantitation kit	UP40840A, 1 kit (500/5000 tests)	UP40840B, 1 kit (125/1250 tests)
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The largest size #UP40840A contains 1L of reagent A, 25mL of reagent B and 10x1mL of standard BSA 2mg/mL

MicroBC Assay protein quantitation kit	UP75860A, 1 kit (500/3400 tests)	UP75860C, 1 kit (50/340 tests)
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The largest size #UP75860A contains 250mL of reagent A, 250mL of reagent B, 12mL of reagent C and 10x1mL of standard BSA



# PRINCIPLE

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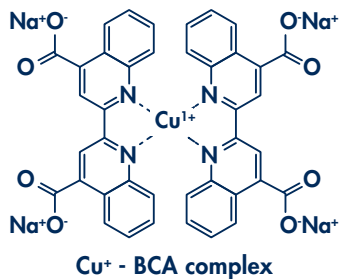
The Bicinchoninic Acid Assay (BC Assay) is a biochemical assay to determine the total level of protein in a solution. Similar to the Bradford protein assay. Total protein concentration is manifested by a change in color of the sample solution from green to purple in proportion to the protein concentration.

## This color change is due to two reactions:

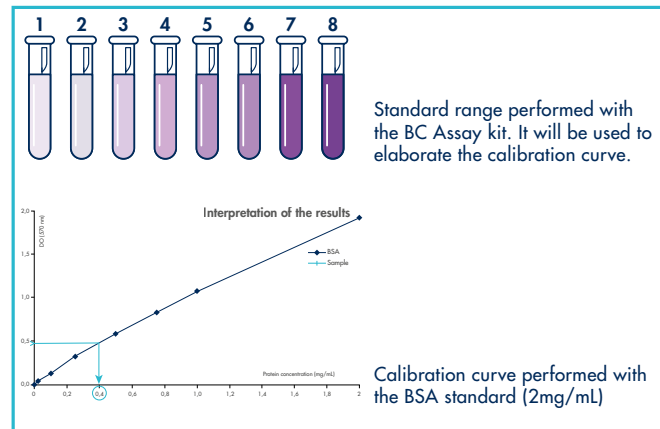
- 1) Peptide bonds in the protein sample reduce  $\text{Cu}^{2+}$  ions, in a temperature dependent reaction. The amount of  $\text{Cu}^{2+}$  reduced is proportional to the amount of protein present in the solution.



- 2) Two molecules of bicinchoninic acid (BCA) chelate with each  $\text{Cu}^+$  ion, forming a violet-colored product that strongly absorbs light at a wavelength of 562 nm.



The amount of protein present in a solution can be quantified by measuring absorption spectra and comparing protein solutions with known concentrations.

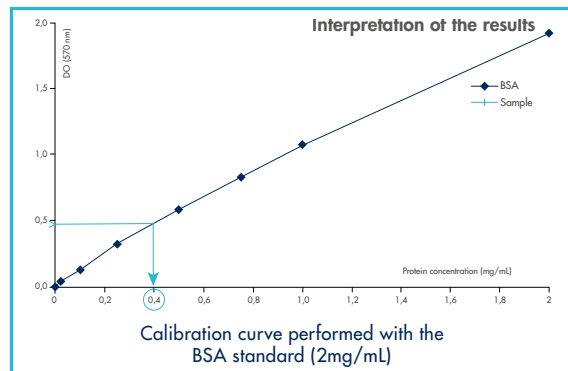


# FEATURES

The technical properties make the BC Assay kit the most popular protein assay in research laboratories. The BC Assay has all the features for the determination of proteins.

- Precise and sensitive colorimetric determination of proteins in solution.
- Working range: 20-2000  $\mu\text{g/mL}$ .
- Ease of use.
- High sensitivity (up to 5  $\mu\text{g/mL}$ ). The detection is done by using a standard Spectrophotometer at 562nm.
- Unequalled linearity.
- Compatibility with many agents such as detergents (SDS), lipids and nucleic acids. It also works perfectly for glycoproteins.

Depending on the desired detection sensitivity, the incubation time with the BCA reagent is adjustable (see page 7). For maximum sensitivity, the MicroBC Assay version allows a protein determination up to 0.5  $\mu\text{g/mL}$ .



BC Assay protein quantitation kit

UP40840A, 1 kit (500/5000 tests)

UP40840B, 1 kit (125/1250 tests)

The largest size #UP40840A contains 1L of reagent A, 25mL of reagent B and 10x1 mL of standard BSA 2mg/mL

MicroBC Assay protein quantitation kit

UP75860A, 1 kit (500/3400 tests)

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The largest size #UP75860A contains 250mL of reagent A, 250mL of reagent B, 12mL of reagent C and 10x1 mL of standard BSA

# MATERIALS NEEDED

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## Materials Supplied



1 vial Reagent A

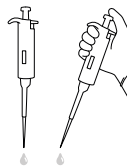


10 vials BSA standard  
(2mg/mL)



1 vial  
Reagent B

## Materials Needed But Not Supplied



Precision pipettes and  
disposable tips



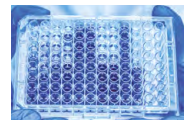
Microtubes  
(1,5mL)



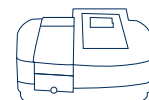
Orbital agitator



Multichannel  
pipettes (300µL)



Microplate  
96 wells



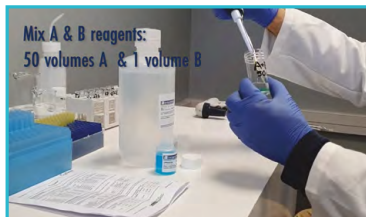
Microplate reader  
(reading at 562nm)



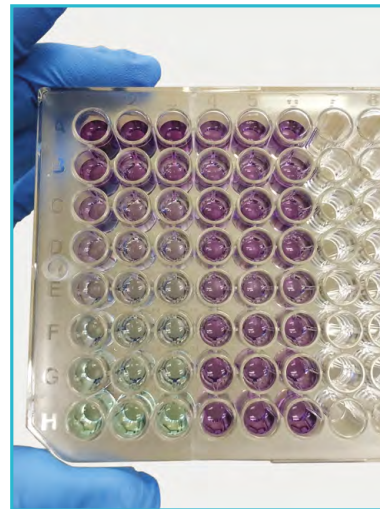
# PROTOCOL: KEY STEPS



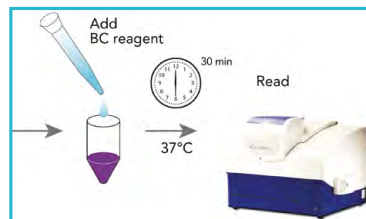
Dilution of standards and samples



Preparation of the BCA Reagent



Microplate distribution of substrates and samples



BCA reagent distribution, incubation and reading at 562nm

For more information, you can watch our video.



SCAN ME



# SENSITIVITY OF THE BC ASSAY

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Depending on the desired detection sensitivity, the dilution ranges and the incubation time with the BCA reagent is adjustable.

## Dilution range to be followed according to the desired sensitivity:



### Standard protocol (20 - 2000µg/mL)

Standard	BSA standard	Water or Buffer	Final protein concentration
Stock Solution	BSA 2mg/mL #UP36859A		
Standard A	300µL of stock	0µL	2mg/mL
Standard B	300µL of stock	300µL	1mg/mL
Standard C	420µL of stock	700µL	750µg/mL
Standard D	100µL of stock	300µL	500µg/mL
Standard E	100µL of stock	700µL	250µg/mL
Standard F	200µL of (E)	300µL	100µg/mL
Standard G	100µL of (F)	400µL	20µg/mL
Blank H	0	700µL	0

### Enhanced protocol (5 - 250µg/mL)

Standard	BSA standard	Water or Buffer	Final protein concentration
Stock Solution	BSA 2mg/mL #UP36859A		
Standard A'	100µL of stock	300µL	500µg/mL
Standard B'	100µL of stock	700µL	250µg/mL
Standard C'	300µL of (B')	300µL	125µg/mL
Standard D'	100µL of (B')	400µL	50µg/mL
Standard E'	48µL of (B')	752µL	15µg/mL
Standard F'	16µL of (B')	784µL	5µg/mL
Standard G'	6µL of (B')	746µL	2µg/mL
Blank H'	0	800µL	0

## Incubation time to be followed according to the desired sensitivity:



Standard protocol	Protocol without heating	Improved protocol
Working range 20µg/mL to 1-2mg/mL	Working range 20µg/mL to 1-2mg/mL	Working range 5-250µg/mL
30 min at 37°C	2 hours at room temperature	30 min at 60°C



# SENSITIVITY OF THE BC ASSAY

**For maximum sensitivity: Use our Micro BCA Assay!**



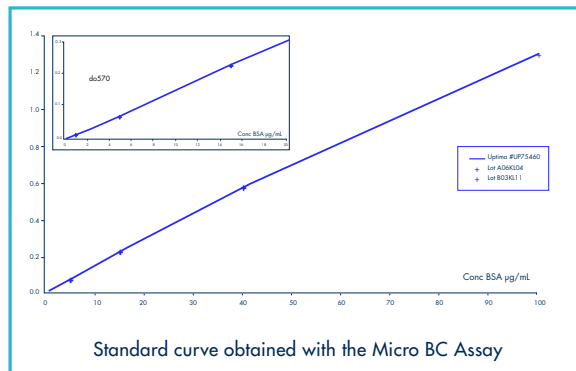
As convenient as BC Assay, but 4 to 6 times more sensitive.  
Working range: 1-200µg/mL

- The Micro BC Assay is high sensitivity up to 0.5µg/mL of protein.
- Incubation 60 min at 37°C or 60°C.

## Uptima™ MicroBC Assay

UP75860A 500-3400 assays (tubes-microplates)

UP75860C 50-340 assays (tubes-microplates)






# COMPATIBLE AND INTERFERING SUBSTANCES:

One of the main advantages of the BC Assay kit is its compatibility with many substances, including detergents and lipids that can interfere with other colorimetric assays such as the Coomassie test.

The following table shows some substances and their compatibility.

Compatible substances (*)	Compatible substances (*)	
Detergents - Good compatibility (*) < 7.5% CHAPS < 5% deoxycholic acid < 5% SDS < 3% Triton X-100 < 6% Tween 20 < 6M Urea	Other - Fairly good compatibility* Carbonate / Bicarbonate pure 100mM < 3mM Glycine HCl pH2.8 < 10mM Glucose < 10% Glycerol < 2M Guanidine HCl < 50mM Imidazole pH7.0 < 150mM MOPS pH7.2 < 1% NaN <sub>3</sub> Pure PBS (0.1M phosphate, 150mM NaCl, pH 7.2) < 1mM PMSF Pure TBS (20mM Tris, 150mM NaCl, pH 7.6) < 0.01% Thimerosal < 50mM TRIS	Other substances  SCAN ME
Solvents - Good compatibility < 20% DMSO < 10% DMF < 15% Methanol < 15% Methanol		
Incompatible substances Creatinine, Cysteine, Tyrosine, Tryptophan Mercaptans Ascorbic acid, EGTA, iron and copper salts H <sub>2</sub> O <sub>2</sub> , hydrazides Phenol red	Reducing agents and agents containing thiols < 0.125mM DTT < 1mM EDTA	

\* A substance is classified as "compatible" if its presence (at a given concentration) does not affect, or modifies by less than 10%, the signal of the BSA standard at 1mg/mL. Compatibility may vary depending on the nature and concentration of the protein to be assayed.

# PROTEIN PREPARATION REAGENT

**Make your BC Assay protein assay kit compatible with any sample by using PPR.**

- Removes non-protein substances present in your samples - i.e. reducing agents, detergents, metals, ...
- Ready to use reagents - ready to use reagents stable at room temperature.
- Four-step protocol, < 10 minutes - operating at room temperature.
- Suitable for processing small samples (up to 50µl).

Protein Preparation Reagent	R5594A, 500 mL
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Contains 250mL of reagent 1 and 250mL of reagent 2

# SELECTION GUIDE

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Method	Detection	Threshold of Detection	Comment	Advion Interchim Scientific Key Products
Bradford	Colorimetric	Very sensitive (2-5µg of proteins)	Colorimetric assay based on the interaction of a dye (Coomassie) with certain amino acids; well known and used but with many procedural modifications. Important protein to protein variations. Very fast method (1-5 min), without incubation and for solutions containing reducing agents	Coo Assay #UPF86400
Bicinchoninic	Colorimetric	High sensitivity	Colorimetric assay, based on the chelation of Cu <sup>2+</sup> ions by bicinchoninic acid with development of an intense purple color. It is the popular standard method, easy to use, reproducible, linear (large working range), low P/P variations, and high compatibility with detergent, lipids, DNA/RNAs and all substances not tolerated by the Lowry method such as reducing agents, chelating agents...	BC Assay #40840A MicroBC Assay #75860A
Biuret	Colorimetric	Low sensitivity (1-20mg)	This assay is based on the reduction of Cu <sup>++</sup> ions under alkaline conditions.	Biuret Assay #1E5350
Lowry	Colorimetric (750nm)	High sensitivity (5-10µg)	Improved Biuret method (Folin-Ciocalteu reagent increases color development) not very easy to use (reagents must be fresh so produced every day; requires 2 incubations; time and temperature must be controlled; possible interference with biological sample components)	Alternatively, the BC Assay is more efficient and more practical
Formazan			Colorimetric assay based on WST-8	Protein Assay Kit #T32790
Epicocconone	Fluorimetric	Very sensitive 40ng/mL	Reversible detection of proteins by binding with this dye. This assay is fully compatible with MS	RED Epicoccostab Fluorescent Assay #FP-CH419A
AccuOrange	Fluorimetric	0.1-15µg/mL	very low P/P variation, but low tolerance to non-ionic detergents unless combined with PPR	AccuOrange Assay #1A8080
Dosage OPA	Fluorimetric	0.1 to 50µg/mL	This highly sensitive assay is especially dedicated to samples containing detergents (SDS, tween 20, tween 80, Brij®-35, CHAPS) and other reducing agents (DTT, β-Mercaptoethanol).	OPA AA/Peptides/Proteins Assay #FP02727A





# Advion Interchim

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**How to request more information, a quotation or to place an order**

Please see from the list below:



## E-mail

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info.EU@advion-interchim.com  
quotes.EU@advion-interchim.com  
orders.EU@advion-interchim.com

## Online

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**[www.advion-interchim.com](http://www.advion-interchim.com)**

## Phone

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**+33 4 70 03 88 55**

**All your orders will be recorded  
& processed as quickly as possible.**

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**You need technical assistance?  
Our scientific experts are here to help.**

**[consumables@advion-interchim.com](mailto:consumables@advion-interchim.com)**

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