

## BCS ASSAY KIT

<u>Code</u>	<u>Description</u>	<u>Size</u>
N962-500RXN	BCS Assay Kit <i>Includes:</i> 50 mL of BCS Assay, Solution A 450 mL of BCS Assay, Solution B Sufficient for 500 Assays	KIT
N962-100RXN	BCS Assay Kit <i>Includes:</i> 10 mL of BCS Assay, Solution A 90 mL of BCS Assay, Solution B Sufficient for 100 Assays	KIT
N962-SAMPLE	BCS Assay Kit <i>Includes:</i> 2 mL of BCS Assay, Solution A 18 mL of BCS Assay, Solution B Sufficient for 20 Assays	KIT

### General Information:

The BCS Assay is a kit that allows for spectrophotometric determination of protein concentration. The BCS Assay utilizes a biuret-like reaction where Copper (II) binds protein. The remaining free Copper (II) then is reduced to Copper (I) which associates with a dye that changes colors upon binding of copper (I). Protein concentration is then determined by measuring the absorbance of the dye-Copper (I) complex. The signal is inversely proportional to the concentration of peptide bonds as opposed to specific amino acid side chains. Therefore, protein to protein variability is less of a concern. Another advantage of the BCS Assay is that the BCS assay has a high tolerance for salts, detergents, and buffers that may interfere with conventional protein quantitation kits.

### Storage/Stability:

Store at 18-26°C

### Application Disclaimer

*For research use only.  
Not for therapeutic or diagnostic use.*

## Procedure

### Standard Curve/Sample Preparation

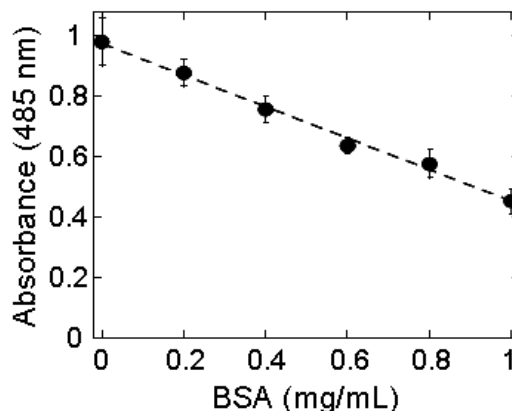
- For optimal results, BSA standards should be prepared in the final storage buffer of the unknown protein preparation.
- Prepare BSA standards according to the table below.

Tube #	Diluent (μL)	20 mg/mL BSA (μL)	Final Concentration (mg/mL)
1	400	0	0
2	396	4	0.2
3	392	8	0.4
4	388	12	0.6
5	384	16	0.8
6	380	20	1

- Aliquot 50 μL of each standard into a 1.5 mL microcentrifuge tube.  
**Note:** To increase accuracy, repeat step 3 two additional times.
- Aliquot 50 μL of the unknown sample into three separate 1.5 mL microcentrifuge tubes.

### Protein Detection

- Add 100 μL of BCS Assay Kit, Solution A to each sample.
- Briefly vortex to mix.
- Add 900 μL of BCS Assay Kit, Solution B to each sample.
- Briefly vortex to mix.
- Measure the absorbance of each sample at 485 nm against water or a diluent reference.
- Construct a standard curve by plotting the absorbances of the BSA standards against their respective concentrations. From this graph, the concentration of the unknown protein preparation can be calculated.



**Figure.** Representative BSA standard curve. The error bars represent the standard deviation obtained from absorbances of three separate samples for each concentration.

Compound	Concentration
Glycerol	≤10% (v/v)
Triton X-100	≤1% (v/v)
Tween-20	<0.2% (v/v)
Nonidet® P-40 Substitute	≤2% (v/v)
EDTA	≤0.1mM
DTT	≤0.3mM

**Table.** BCS Assay reagent compatibility. For protein preparation storage buffers containing reagents above the concentrations mentioned, the BSA standards should be prepared with storage buffer. Alternatively, the unknown protein preparation can be diluted to minimize the affects of the reagents mentioned in the table.

## Related Products

Code	Product
J642-1ML	Bovine Serum Albumin,
J642-5ML	20mg/mL
K763-KIT	BSA Standards Kit
0254-500ML	Acryl/Bis 37.5:1 (30:0.8)
N836-KIT	Protein EZ-Vision®, 4X

www.interchim.com



211 bis Avenue Kennedy - BP 1140  
03103 Montluçon - France  
33 (0) 4 70 03 88 55  
Fax 33 (0) 4 70 03 82 60  
e-mail [interchim@interchim.com](mailto:interchim@interchim.com)

Agence Paris - Normandie  
33 (0) 1 41 32 34 40  
Fax 33 (0) 1 47 91 23 90  
e-mail [interchim.paris@interchim.com](mailto:interchim.paris@interchim.com)

## References

1. Matsushita M., Irino T., Komoda T., and Sakagishi Y. (1993) Determination of proteins by a reverse biuret method combined with the copper-bathocuproine chelate reaction. Clinica Chimica Acta, **216**, 103-11

## Frequently Asked Questions

Questions	Answers
Why does my 0 mg/mL BSA standard read <0.9 OD?	<ul style="list-style-type: none"><li>• Chelating agents such as EDTA and reducing agents such as DTT found in protein storage or dilution buffers interfere with the quantitation.</li></ul>
Why do my standard curve absorbance results differ from the manufacturer's standard curve?	<ul style="list-style-type: none"><li>• The BSA standards were not prepared as described</li><li>• The diluent for the BSA standards contains chemicals that interfere with the quantitation.</li></ul>

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211 bis Avenue Kennedy - BP 1140  
03103 Montluçon - France  
33 (0) 4 70 03 88 55  
Fax 33 (0) 4 70 03 82 60  
e-mail [interchim@interchim.com](mailto:interchim@interchim.com)

Agence Paris - Normandie  
33 (0) 1 41 32 34 40  
Fax 33 (0) 1 47 91 23 90  
e-mail [interchim.paris@interchim.com](mailto:interchim.paris@interchim.com)