

Miniature protein quantification in IMAPlate with BCA assay

INTRODUCTION: The bicinchoninic acid assay (BCA assay) is a biochemical assay for determining the total level of protein in a solution. The BCA assay is based on the reduction of Cu^{2+} ions from the cupric sulfate to Cu^{1+} ions that are then chelated by bicinchoninic acid to form a purple-colored product that strongly absorbs light at a wavelength of 562 nm. The amount of reduced Cu^{2+} ions is proportional to the amount of protein present in the solution. At lower temperatures the color development is the result of the interaction of copper and BCA with cysteine, cystine, tryptophan, and tyrosine residues in the protein. At elevated temperatures, the peptide bond is also responsible for color development.

The IMAPlate 5RC96, as a versatile lab device, can be used as micro volume long light path-length 96-well plate for any kind of colorimetric homogeneous assays. Performing BCA assay in IMAPlate 5RC96 follows the same reaction conditions of 96-well microplate procedure supplied in commercial BCA assay kit except that the volume of the reagent and sample solution can be reduced to one-tenth (**1/10 VOLUME**). Although the sample volume is reduced down to **2.5 μl** from 25 μl , the sensitivity of this modified procedure of BCA assay in IMAPlate 5RC96 still keeps the same as the microplate procedure. Therefore, the IMAPlate 5RC96 BCA assay procedure provides a miniature protein quantification method with minimized sample solution.

ASSAY PROCEDURE: *(follow the same reaction condition of 96-well microplate procedure provided in the Pierce BCA protein assay kit)*

Seal the bottom of IMAPlate with a piece of parafilm



Pipette 2.5 μl of standards or samples to the upper compartment



Pipette 20 μl fresh prepared working reagent solution to the standards and samples



Mix thoroughly with a plate shaker for 30 seconds



Incubate at 37°C 30 minutes



Cool the plate to room temperature



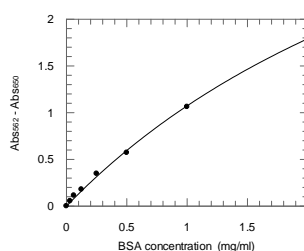
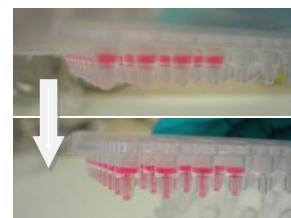
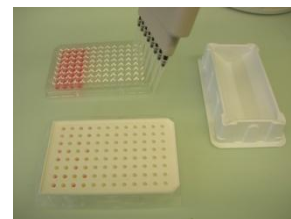
Peel off the parafilm and gently tap the IMAPlate in horizontal direction several times to ensure the assay solution filling up the lower compartment and place in an IMAPlate adaptor.



Measure the peak absorbance at 562nm and base-line absorbance at 650nm on a plate reader.



Use (Abs562 – Abs650) to plot the standard curve and to determine the concentration of unknown samples.



RESULTS & DISSCUSION:

Table 1 lists the absorbance values of a set of two-fold serial diluted standards and an unknown sample obtained from BCA assay performed in 96-well plate with the microplate procedure of the BCA protein assay kit from Pierce (Thermo Scientific) and in IMAPlate 5RC96 with the modified BCA assay procedure described above. The absorbance of all standards and sample obtained from IMAPlate 5RC96 is slightly higher than that from 96-well plate correspondently, which is expected due to a longer light path-length of IMAPlate with 22.5 μ l of assay solution than that of 96-well plate with 225 μ l of assay solution.

Figure 1 plots the data in table 1 and their best fitting curves derived with the one site binding mode of GraphFit software. Both sets of data are well distributed around their best fitting curves and follow the theoretic binding mode. The concentration of the unknown sample determined in these two systems is very similar, 0.625 mg/ml in IMAPlate 5RC96 vs 0.649 mg/ml in 96-well plate.

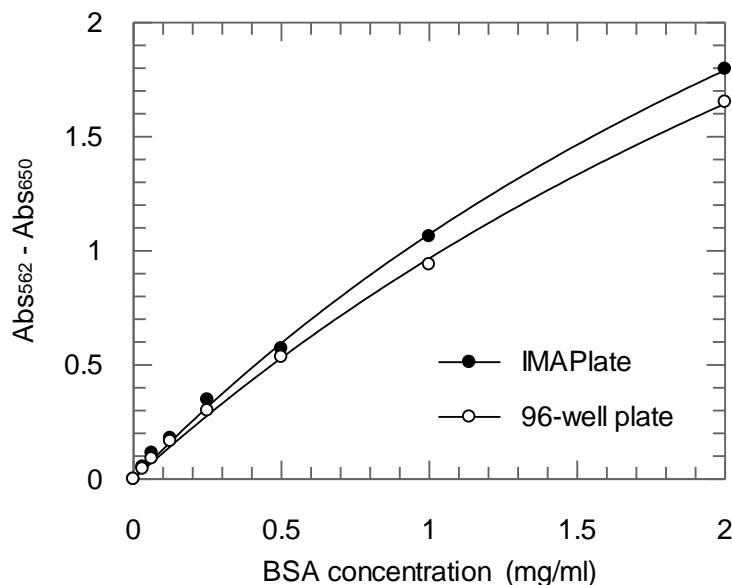


Figure 1. BCA protein assay best fitting standard curves from IMAPlate and 96-well plate.

Table 1. Absorbance values of BCA assay form 96-well microplate and IMAPlate 5RC96

	mg/ml	96-well microplate				IMAPlate 5RC96			
		Abs562-Abs650	Mean	SD	CV%	Abs562-Abs650	Mean	SD	CV%
St01	2	1.684	1.652	0.046	2.8	1.747	1.796	0.069	3.9
		1.619				1.845			
St02	1	0.961	0.940	0.030	3.2	1.083	1.062	0.029	2.8
		0.918				1.041			
St03	0.5	0.548	0.534	0.020	3.7	0.584	0.572	0.018	3.1
		0.520				0.559			
St04	0.25	0.305	0.300	0.007	2.4	0.364	0.347	0.024	6.9
		0.295				0.330			
St05	0.125	0.167	0.165	0.003	1.6	0.176	0.179	0.005	2.6
		0.163				0.182			
St06	0.063	0.089	0.089	0.001	1.0	0.113	0.114	0.002	1.4
		0.088				0.115			
St07	0.031	0.046	0.045	0.001	1.9	0.057	0.053	0.005	8.6
		0.045				0.050			
Sample	unknown	0.672	0.673	0.001	0.2	0.709	0.722	0.018	2.5
		0.674				0.735			