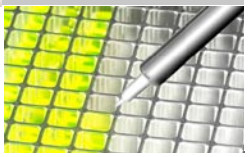

Determination of protein concentrations with Varioskan™ using BCA protein assay

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This paper describes the use of Thermo's Varioskan spectrophotometer and spectrofluorometer for protein concentration determinations using BCA protein assay and photometric detection. The measurement wavelength was selected based on the spectral scanning option of the Varioskan and the wavelength giving maximum absorbance was used for

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Abstract

Photometric assays are commonly used in many laboratories for protein detection as they are well established, reliable and inexpensive to run. BCA protein assay in one of the most popular methods for colorimetric detection and quantification of total protein (Smith et al. 1985). The assay is based on the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective photometric detection of cuprous cation (Cu^{1+}) by bicinchoninic acid. The purple-colored reaction product is formed as a result of the molecules of BCA with one Cu^{1+} ion.

Experimental

The protein concentration was determined from purified recombinant testosterone binding antibody fragments produced in *E. Coli* (Skerra and Pluckthun, 1988). Either wild type or biotinylated wild type (wt or wt-biot) as well as mutated fragments (mut3, mut8, mut3+8, 77-biot) have been used for concentration determination.

The protein concentration determination was performed using BCA Protein Assay Kit (Pierce Biotechnology Inc, Rockford, IL) according to manufacturer instructions. BSA standard (included in the kit) was used to prepare a standard curve between 0.0313 - 1.0 mg/ml. Two replicates of purified antibody fragments were measured as samples. Both standards and samples were diluted in PBS buffer (15 mM P_i – 150 mM NaCl, pH 7.4). Clear 96-well microtiter plates (Thermo Microtiter®) were used for all measurements.

Before the actual protein concentration measurement, the optimal wavelength for BCA assay was determined using Varioskan photometric spectral scanning option between wavelengths 280 - 580 nm. Spectra of standard curve samples 0 mg/ml (blank) and 1.0 mg/ml were determined and the resulting spectra are shown in Figure 1. (Only the relevant wavelength area between 450 - 580 nm is shown in the figure.)

Before the photometric measurement the sample plate was mixed for 2 min at RT and incubated in 37 °C for 1 hour without shaking. The measurements were performed at 560 nm. This wavelength was selected based on the wavelength optimization because it is producing the maximum absorbance (Figure 1.). As a reference, the measurements were also performed at 540 nm wavelength, as it is the most common filter used for BCA assay with filter based photometers. According to kit instructions, the recommended wavelength to measure BCA assay is 562 nm although wavelengths from 540-590 nm have been used successfully with this method.

Results and Discussion

The absorption maximum for BCA assay was 560 nm according to Varioskan spectral scanning measurement which is practically the same as recommended in Pierce BCA Protein Assay Kit (562 nm).

The protein concentration determination with Thermo Varioskan results practically into the same concentration levels with both measured wavelengths (560 and 540 nm), see Figures 2 and 3. This is understandable as the spectrum of BCA color is rather wide as one can see from the Figure 1.

These results show that Varioskan can easily be used in protein concentration determination based on BCA assay. All assay steps (shaking, incubation, measurement) can easily be adapted in one single SkanIt™ software protocol. In addition, when using microplates and microplate reader like Varioskan, more samples can be analyzed in less time, sample handling is minimized and reagent usage is reduced.

References

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985). Measurement of protein using bicinchoninic acid. Anal Biochem. 150:76-85.

Skerra A, Pluckthun A. (1988). Assembly of a functional immunoglobulin Fv fragment in Escherichia coli. Science. 240(4855):1038-41.

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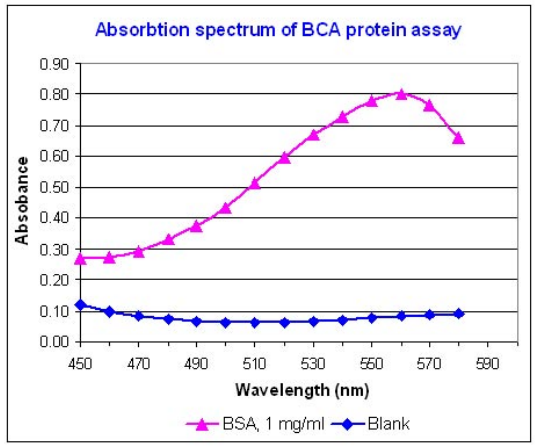

Figure 1. Absorbance spectrum of BCA color reaction. The absorption maximum was determined to be 560 nm.

BCA standard curve at 560 nm					
BSA (mg/ml)	sample 1	sample 2	Average	Sample - Blank	
0.0	0.084	0.080	0.082	0.000	
0.0313	0.086	0.081	0.084	0.001	
0.0625	0.110	0.107	0.109	0.027	
0.125	0.162	0.159	0.161	0.079	
0.25	0.268	0.265	0.267	0.185	
0.50	0.465	0.458	0.462	0.380	
1.0	0.812	0.810	0.811	0.729	
Purified Fab fragments					Protein (mg/ml)
77	0.712	0.700	0.706	0.624	0.82
wt	0.672	0.685	0.679	0.597	0.78
mut3	0.416	0.421	0.419	0.337	0.44
mut8	0.515	0.505	0.510	0.428	0.56
mut 3+8	0.201	0.199	0.200	0.118	0.14
77-biot	0.911	0.899	0.905	0.823	1.09
wt-biot	0.672	0.671	0.672	0.590	0.77

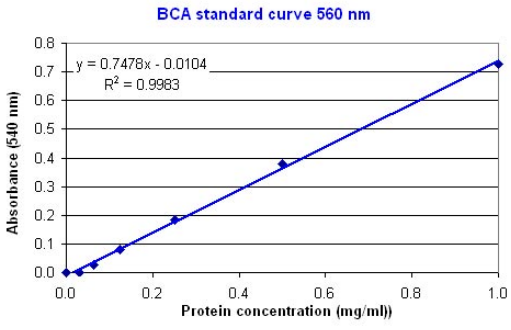

Figure 2. BCA standard curve and calculated antibody fragment concentrations measured with Varioskan at 560 nm.

BCA standard curve at 540 nm					
BSA (mg/ml)	sample 1	sample 2	Average	Sample - Blank	
0.0	0.079	0.074	0.077	0.000	
0.0313	0.080	0.077	0.079	0.002	
0.0625	0.106	0.102	0.104	0.028	
0.125	0.158	0.156	0.157	0.081	
0.25	0.264	0.261	0.263	0.186	
0.50	0.459	0.451	0.455	0.379	
1.0	0.799	0.792	0.796	0.719	
Purified Fab fragments					Protein (mg/ml)
77	0.706	0.697	0.702	0.625	0.84
wt	0.667	0.677	0.672	0.596	0.80
mut3	0.407	0.412	0.410	0.333	0.44
mut8	0.505	0.495	0.500	0.424	0.56
mut 3+8	0.196	0.195	0.196	0.119	0.15
77-biot	0.889	0.879	0.884	0.808	1.08
wt-biot	0.654	0.657	0.656	0.579	0.77

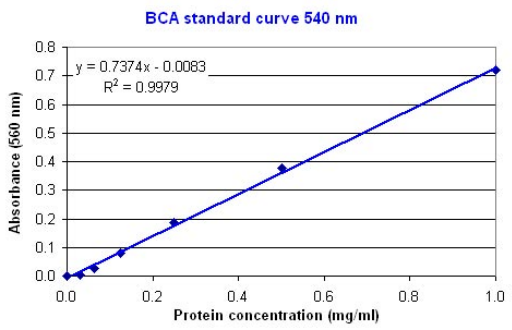

Figure 3. BCA standard curve and calculated antibody fragment concentrations measured with Varioskan at 540 nm

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