



Comparison of mouse spleen protein quantification by PiCOEXPLORER and a microplate reader

Background

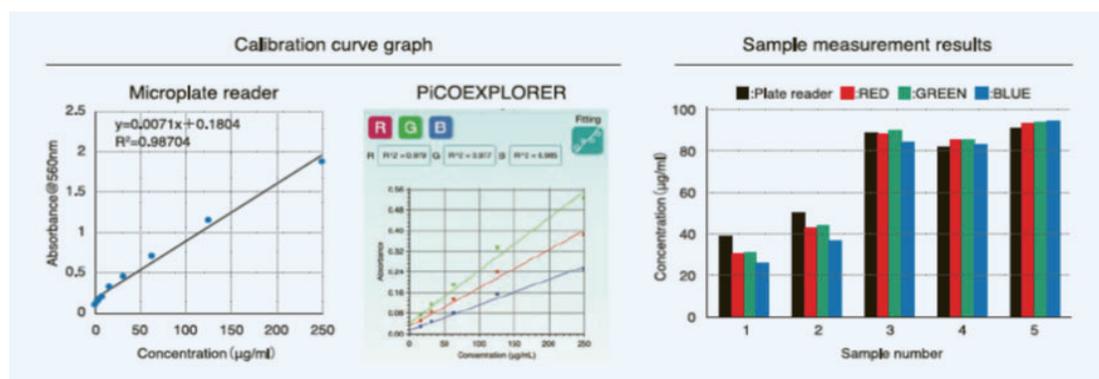
The bicinchoninic acid assay is a method for quantifying proteins in an alkaline solution by observing how the color of the solution changes to purple as divalent copper ions (Cu^{2+}) are reduced to monovalent copper ions (Cu^+) by the protein, with the color change occurring as the monovalent copper ions each combine through coordinate bonding with two molecules of bicinchoninic acid (BCA).

This application note describes the use of a microplate reader (measurement wavelength: 560 nm, Infinite F200; Tecan, Japan) and the PiCOEXPLORER (color sensor: R/G/B) for measuring concentration when assaying protein extracted from the spleen of autoimmune disease model mice ($n=5$) using the BCA method (Micro BCA Protein Assay Kit, Thermo Scientific).

Experiment Conditions

Reaction liquid volume: Plate reader, 200 μl , PiCOEXPLORER, 40 μl

Result



The plate reader used in this experiment measured absorption at 560 nm to prepare the calibration curve and quantify protein. Sample concentration measurement results obtained with the PiCOEXPLORER using the RED (575–660 nm wavelength region) and GREEN (455–630 nm wavelength region) color sensors compared well to those of the plate reader. Measurement values between the two instruments tended to be closer at higher sample concentrations. The absorption wavelength recommended for measurement with the Micro BCA Protein Assay Kit is 562 nm, so the GREEN sensor (455–630 nm) is probably most suitable for measurement, but there was no great difference in the R^2 and concentration values obtained with the RED sensor, so it is also probably useful for measurement.

Comments

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Formerly, I had to prepare calibration curves and calculate sample concentrations using Excel etc., but the PiCOEXPLORER can prepare calibration curves automatically, and samples can be quantified in just 1 or 2 seconds. This is extremely useful. I also like it that the minimum sample volume required for measurement is just 30 μl . Calibration curves are prepared based on a maximum of five known concentration data points, so I'm looking forward to see how many points can be added for higher accuracy.