APPLICATION NOTE

Measure total protein in cell lysates with SpectraMax ABS Plus Microplate Reader

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Benefits

- **Accurately assay protein concentrations with minimal incubation time**
- **Obtain results quickly with the 8-channel read head**
- **Plot standard curves and calculate sample protein concentrations automatically with preconfigured protocols in SoftMax Pro Software**

Introduction

Quantification of protein concentrations from cell lysates is a key step for many downstream applications, such as western blots and enzyme-linked immunosorbent assays (ELISAs). Two of the most popular assays are colorimetric, relying on a color change in a reagent in the presence of protein. The Bradford protein assay is based on the absorbance shift in Coomassie blue dye from 465 nm (unbound) to 595 nm (bound). The bicinchoninic acid assay (BCA) relies on two reactions: (1) protein reduces Cu^{2+} to Cu^{1+} and (2) bicinchoninic acid binds to Cu^{1+}, forming a purple-colored complex that strongly absorbs light at 562 nm.

Here we describe the use of the Pierce Rapid Gold BCA and Pierce Detergent Compatible Bradford assays to quantitate total protein in cell lysates. The standard BCA assay requires a 30-minute incubation at 37°C, but the Rapid Gold BCA assay only requires a 5-minute incubation at room temperature, and absorbance is measured at 480 nm. The detergent compatible Bradford assay overcomes problems encountered when Coomassie dye is used with cells lysed in buffers containing detergents.

Both assays were read on the SpectraMax® ABS Plus Microplate Reader. Preconfigured protocols in SoftMax® Pro Software were used to acquire data and plot standard curves from which the concentrations of cell lysates were determined. Both the Rapid Gold BCA assay and the detergent compatible Bradford assay gave similar results with the cell lysates used.

Materials

- Pierce Rapid Gold BCA Protein Assay Kit (ThermoFisher cat. #A53226)
- Pierce Detergent Compatible Bradford Assay Kit (ThermoFisher cat. #23246)
- RIPA Buffer (ThermoFisher cat. #89900)
- Halt Protease Inhibitor Single-Use Cocktail (ThermoFisher cat. #78430)
- Greiner clear 96-well microplate (Greiner Bio-One cat. #655101)
- CHO-K1 cells (ATCC cat. #CCL-61)
- SpectraMax ABS Plus Microplate Reader (Molecular Devices cat. #ABS Plus)

Note – For the assays described here, similar results are obtained with the SpectraMax ABS and SpectraMax PLUS 384 Microplate Readers

Methods

Cell lysate preparation

Media was aspirated from a T-75 flask of CHO-K1 cells, and cells were washed with 10 mL of cold PBS with calcium and magnesium. After PBS was aspirated, 2 mL of RIPA buffer was added to the flask, and cells were incubated for 5 minutes at room temperature to ensure complete lysis. Halt protease inhibitor was added to the lysate at a final concentration of 1X. The lysate was divided and transferred into two 1.5 mL microcentrifuge tubes and centrifuged at 14,000 rpm for 20 minutes. A 1:3 dilution series of the supernatant was made in PBS to test performance of the assays over a range of cell and lysis buffer concentrations.

Standard curves

For both protein assays, bovine serum albumin (BSA) standards ranging from 25 µg/mL to 2000 µg/mL were prepared according to the assay kit instructions.

Rapid Gold BCA assay

20 µL of BSA standard or cell lysate was pipetted into wells of a 96-well microplate (n = 3), followed by 200 µL of Working Reagent (50:1 Reagent A:Reagent B). The plate was placed on a shaker for 30 seconds to mix contents, and then incubated for five minutes at room temperature without shaking. Absorbance was read at 480 nm on the SpectraMax ABS Plus reader.

Detergent Compatible Bradford assay

10 µL of BSA standard or cell lysate was pipetted into wells of a 96-well microplate (n = 3). 300 µL of Detergent Compatible Bradford reagent was added to each well. Contents of wells were pipetted up and down several times to mix. The plate was incubated for 10 minutes at room temperature and read at 595 nm on the SpectraMax ABS Plus reader.

Data analysis

Standard curves were plotted using the quadratic curve fit in SoftMax Pro Software, and the concentrations of cell lysates were calculated from the standard curves by the software.

Results

BSA standard curves for the Rapid Gold BCA and Detergent Compatible Bradford assays are shown in Figure 1. SoftMax Pro Software automatically calculated cell lysate concentrations from the standard curve for each assay.

Figure 2 shows the data table obtained for the Rapid Gold BCA assay, with concentrations calculated for each dilution of the cell lysate. The average concentration of cell lysate was calculated to be 683 µg/mL. Figure 3 shows the data table for the Detergent Compatible Bradford assay with average concentration calculated to be 673 µg/mL, similar to the BCA assay result.

Conclusion

The Pierce Rapid Gold BCA and Pierce Detergent Compatible protein assays provide users easy methods for quantification of total protein in cell lysate samples without long incubation times or worries over the effects of detergent in lysis buffers. The Rapid Gold BCA assay yielded a more linear standard curve and more consistent results over a range of diluted cell lysates, but overall results were similar for both kits.

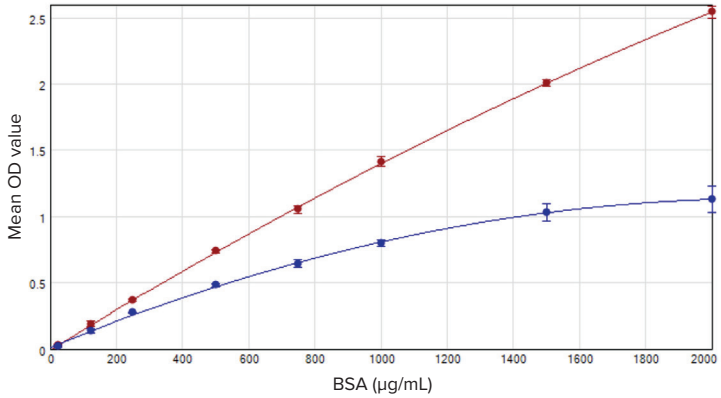

Figure 1. BSA standard curves for the BCA Rapid Gold assay (red plot, r^2 = 1.000) and Detergent Compatible Bradford assay (blue plot, r^2 = 0.999).

Sample	Wells	Dilution Factor	OD Value	R	Concentration	MeanConc	SD	CV	AdjConc
01	E1	1.000	0.355		239.916	401.514	252.166	62.8	401.514
	F1		0.996		692.080				
	G1		0.403		272.547				
02	E2	3.000	0.395		266.970	242.004	24.479	10.1	726.011
	F2		0.356		240.998				
	G2		0.322		218.043				
03	E3	9.000	0.151		103.796	90.176	12.044	13.4	811.580
	F3		0.124		85.800				
	G3		0.116		80.931				
04	E4	27.000	0.044		33.386	29.410	4.630	15.7	794.073
	F4		0.039		30.517				
	G4		0.030		24.327				

Mean Adjusted Result: 683.3

Figure 2. Rapid Gold BCA assay results. SoftMax Pro Software data table shows the calculated concentrations of serially diluted cell lysate.

Sample	Wells	Dilution Factor	OD Value	R	Concentration	MeanConc	SD	CV	AdjConc
01	E1	1.000	0.224		216.035	203.674	21.969	10.8	203.674
	F1		0.225		216.679				
	G1		0.188		178.309				
02	E2	3.000	0.217		208.432	223.730	19.160	8.6	671.190
	F2		0.225		217.537				
	G2		0.251		245.221				
03	E3	9.000	0.117		104.006	98.806	4.895	5.0	889.258
	F3		0.111		98.128				
	G3		0.107		94.286				
04	E4	27.000	0.050		37.286	34.344	6.235	18.2	927.291
	F4		0.051		38.564				
	G4		0.039		27.182				

Mean Adjusted Result: 672.9

Figure 3. Bradford assay results. SoftMax Pro Software data table shows calculated concentrations of cell lysate with the Detergent Compatible Bradford assay.

The SpectraMax ABS Plus reader with SoftMax Pro Software offers a complete solution for data acquisition and analysis, with preconfigured protocols that plot standard curves and calculate sample concentrations automatically. With the Speed Read setting, a 96-well plate can be read in just five seconds, further streamlining the process of protein quantification.

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