APPLICATION NOTE

Streamline BCA-based protein quantitation on the SpectraMax iD5 reader

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Benefits

- **Quantitate proteins rapidly with a 5-minute, room-temperature incubation**
- **Measure assay absorbance values quickly with minimal background noise on the SpectraMax iD5 reader**
- **Graph standard curves and calculate sample protein concentrations easily with a preconfigured protocol in SoftMax Pro Software**

Introduction

The BCA assay is a two-step colorimetric assay used to quantitate the total protein in a sample. This assay uses the Biuret reaction, in which Cu^{2+} is reduced to Cu^{1+} by protein in an alkaline medium. The amino acid backbone forms a color-chelate complex with the copper molecules, allowing for a highly sensitive and selective detection of the cuprous cation. The resulting water-soluble complex exhibits strong absorbance which can be measured on a microplate reader. The original Pierce™ BCA Protein Assay Kit requires incubation for 30 minutes at 37°C and produces a color change that is measured at 562 nm. A newer version of the assay, the Pierce Rapid Gold BCA Protein Assay Kit, simply requires incubation for five minutes at room temperature and yields an intense orange-gold color that is measured at 480 nm.

The SpectraMax® iD5 Multi-Mode Microplate Reader includes an absorbance detection mode optimal for protein quantitation assays. Here, we illustrate how the SpectraMax iD5 reader, in combination with SoftMax® Pro Software, is used to quantitate a cellular protein sample with two different kits, Pierce BCA Protein Assay Kit and Pierce Rapid Gold BCA Protein Assay Kit. The kits include bovine serum albumin, which is used to set up a standard curve from which the concentrations of samples are interpolated and reported automatically by a preconfigured protocol in SoftMax Pro Software. The 5-minute incubation at room temperature used for the Rapid Gold BCA assay, compared to the 30-minute incubation at 37°C required for the original kit, offers time savings and convenience.

Materials

- SpectraMax iD5 Multi-Mode Microplate Reader (Molecular Devices cat. #ID5-STD)
- Pierce BCA Protein Assay Kit (ThermoFisher Scientific cat. #23225)
- Pierce Rapid Gold BCA Protein Assay Kit (ThermoFisher Scientific cat. #A53225)
- RIPA Buffer (ThermoFisher Scientific cat. #89900)
- Halt Protease Inhibitor Single-Use Cocktail (ThermoFisher Scientific cat. #78430)
- 96-well, clear, flat-bottom polystyrene microplate (Greiner Bio-One cat. #655101)
- 293 [HEK-293] cells (ATCC cat. #CRL-1573)

Methods

HEK-293 cells were cultured in a T75 culture flask until sub-confluent. Cells were then washed with 10 mLs of cold phosphate-buffered saline containing calcium and magnesium (PBS). 2 mLs of cold RIPA buffer were added to the flask and remained on the cells for five minutes, then lysed cells were pipetted up and down several times to release any remaining cellular material from the flask surface. The lysate was divided into two 1.5-mL microcentrifuge tubes, and Halt Protease Inhibitor was added. Lysates were centrifuged at 15,000 rpm for 20 minutes, and supernatant was used to make a 3-fold dilution series of the unknown sample in PBS. A dilution series was performed to ensure that some sample dilutions would fall within the assay's measurable range.

BSA standard curves were prepared by following the method given in each kit's user guide. 12 mL of each BCA working reagent were prepared using a 50:1 ratio of Reagent A to Reagent B. 25 µL of protein standards or cell sample were transferred in triplicate (one set per assay) to clear 96-well microplates. One plate received 200 µL of the BCA working reagent, and the other received 200 µL of the Rapid Gold BCA working reagent. The BCA assay plate was incubated for 30 minutes at 37°C, and the BCA Rapid Gold assay plate was incubated for five minutes at room temperature.

Absorbance values were read on the SpectraMax iD5 reader using a preconfigured BCA assay protocol (the SpectraMax iD3 reader is also suitable for these assays, as it has the same absorbance performance as the SpectraMax iD5 reader). The BCA assay plate was read at 562 nm, the original wavelength setting in the protocol, while for the BCA Rapid Gold assay the wavelength setting was changed to 480 nm prior to reading the plate.

BSA standard curves were generated by SoftMax Pro Software, and a quadratic curve fit was applied, as recommended in the assay kit user guides. The standard curve for each assay was used to calculate the concentrations of the HEK-293 cell samples. Refer to Figure 1 for a visual representation of the workflow for each kit.

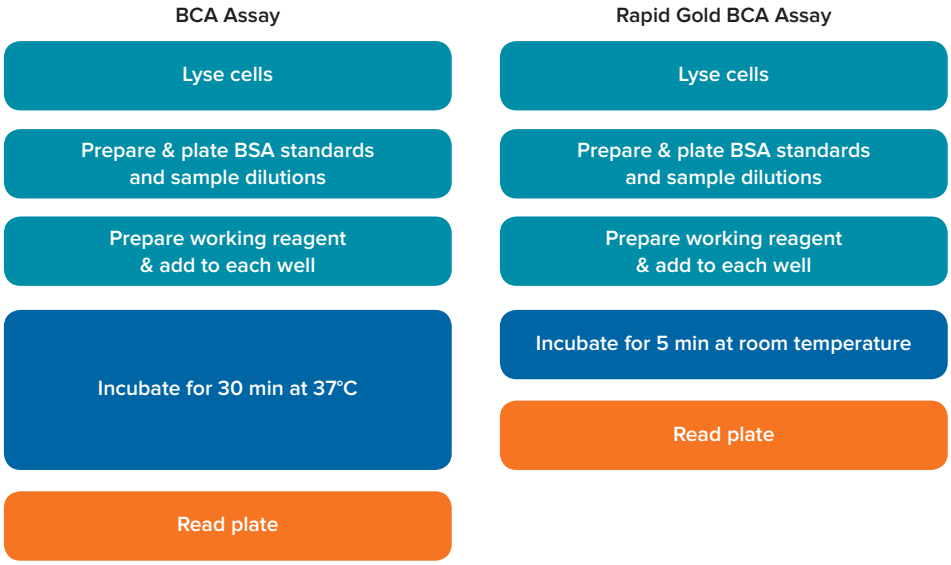

Figure 1. Comparison of assay workflow. Steps in the experimental procedures for the BCA assay and Rapid Gold BCA assay are depicted. The BCA assay requires an incubation of 30 minutes at 37°C, while the Rapid Gold BCA assay only requires a 5-minute incubation at room temperature.

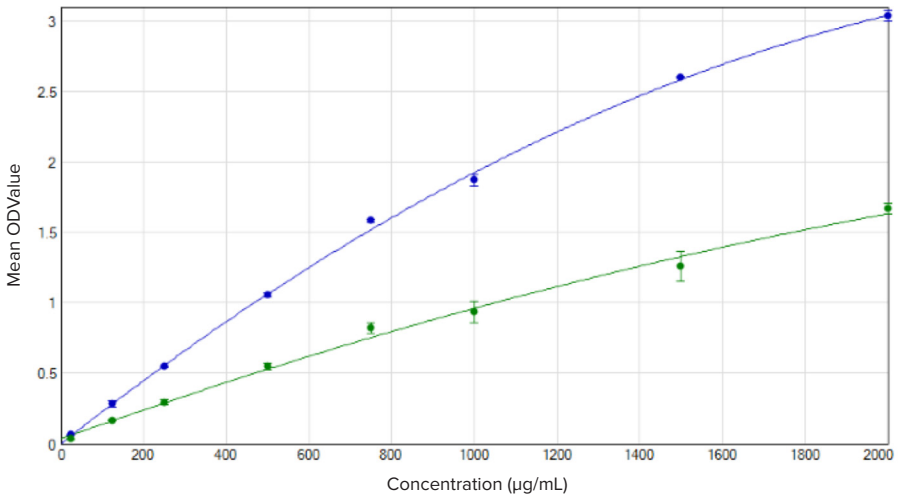

Figure 2. BSA standard curves. Standard curves were plotted using the quadratic curve fit in SoftMax Pro Software for the BCA (green) and BCA Rapid Gold (blue) assays. For BCA, $r^2 = 0.997$, and for BCA Rapid Gold, $r^2 = 0.999$. For each curve, n=3.

Results

The BSA standard curves for the BCA assay and Rapid Gold BCA assay both yielded r^2 values above 0.99 using a quadratic curve fit (Figure 2). The range of OD values seen from low to high BSA standard was larger for the BCA Rapid Gold assay than for the BCA assay.

Cell lysate concentrations were interpolated from the standard curves, calculated automatically by SoftMax Pro Software, and displayed in a group table (BCA Rapid Gold group table is shown in Table 1). For the BCA Rapid Gold assay, the original cell lysate sample concentration was found to be 680 µg/mL while for the BCA assay it was about 640 µg/mL, thus similar concentrations were calculated despite the difference in OD ranges observed between the two standard curves.

Conclusion

The BCA Rapid Gold assay enables microplate incubation at room temperature for a total of five minutes, compared to the original BCA assay, which requires the microplate to be incubated at 37°C for 30 minutes. Both assays yielded standard curves with excellent r^2 values and similar calculated sample concentrations.

The SpectraMax iD5 reader rapidly performs absorbance measurements that are used to plot standard curves and calculate sample concentrations using a preconfigured protocol in SoftMax Pro Software. The protocol contains settings for reading the plate, as well as template groups that allow a user to easily set up analysis, including calculation of sample concentrations from a standard curve.

Unk_Dilution

Sample	Wells	OD_Values	R	Concentration	MeanConc	SD	Dilution	AdjConc
01	B4	0.838		386	419	121.8	1.0	419
	B5	1.166		554				
	B6	0.698		317				
02	C4	0.486		217	209	33.4	3.0	626
	C5	0.529		237				
	C6	0.388		172				
03	D4	0.192		84	76	9.4	9.0	684
	D5	0.179		78				
	D6	0.151		66				
04	E4	0.056	R	24	25	3.4	27.0	679
	E5	0.066		29				
	E6	0.051	R	22				

Table 1. Example of a group table for the BCA Rapid Gold assay. For each OD value measured, a concentration was interpolated from the standard curve. OD values falling outside the range covered by the standard were flagged as 'R'. For each mean concentration, an adjusted concentration was calculated by multiplying the mean concentration by the dilution factor of the sample. Samples 02, 03, and 04 gave consistent adjusted concentrations, so the original sample's concentration was estimated to be 680 µg/mL.

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