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In-situ Micro-Volume BCA Assay
Using Epoch™ & Take3™ Multi-Volume Plate

This Tech Note describes the materials, methods and Gen5™ parameters used to perform an in-situ micro-volume based bicinchoninic acid (BCA) assay using the Take3 plate and Epoch reader. For more general information regarding Gen5™ Data Analysis Software (e.g. general concepts, data analysis, etc.) please refer to the software's help system. Be sure to thoroughly read the application note titled *In-situ Micro-Volume Bicinchoninic Acid Protein Assay* on www.biotek.com.

Materials Used:

- Bicinchoninic Acid Protein Assay Kit (Sigma, PN-BCA1 and B9643)
- Purified water (like MilliQ™)
- Single- and 8-channel pipettor with 1-10 µL range

Assay Setup:

- The BCA Working Reagent was prepared just prior to use as per the manufacturer's recommendations.
- A 6 point 1:3 serial dilution series of protein standards was creating from a stock solution of BSA at 1 mg/mL (Sigma, PN-3294) in MilliQ™ water. First, 2 µL of protein standards and samples were sequentially loaded directly onto the Take3 microspots, followed by 2 µL of BCA Working Reagent (using an 8-channel pipettor with mixing).
- The blanks were made of a 1:1 volume ratio BCA working reagent and MilliQ™ water.
- The reaction was incubated at room temperature (~22ºC) for 25 minutes then read on the Epoch™ Microplate Reader.
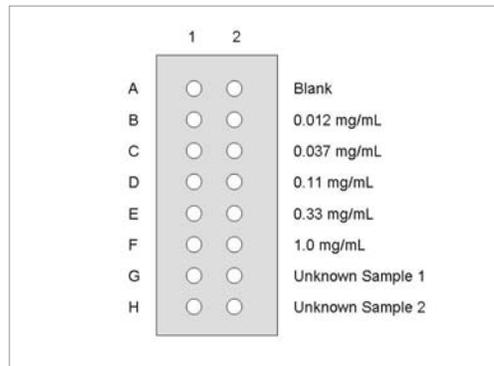
Epoch & Gen5/Take3 Setup:

- Ensure that the Take3 plate is defined in the Gen5 Take3 Module
- With the Take3 plate defined, create a standard Gen5 protocol to run this assay. See details below.

Gen5 Recommended Protocol Setup:

1. Select the Take3 plate from the Plate Type dropdown
2. Read step: 562 nm, Normal speed, wells A2->H3
3. The example Plate Layout is shown below, using the well locations A2-H3, corresponding to the microspots of the Take3 plate:

Plate Layout

Well Settings

Type: Standard

ID Prefix: STD

Conc.: 0

	1	2	3	4
A		STD1 0	STD1 0	
B		STD2 0.012	STD2 0.012	
C		STD3 0.037	STD3 0.037	
D		STD4 0.11	STD4 0.11	
E		STD5 0.33	STD5 0.33	
F		STD6 1	STD6 1	
G		SPL1	SPL1	
H		SPL2	SPL2	

	1	2	
A	○	○	Blank
B	○	○	0.012 mg/mL
C	○	○	0.037 mg/mL
D	○	○	0.11 mg/mL
E	○	○	0.33 mg/mL
F	○	○	1.0 mg/mL
G	○	○	Unknown Sample 1
H	○	○	Unknown Sample 2

4. Data Reduction steps required:

Transformation

Data In: 562

[Select multiple data sets...]

New Data Set Name: pathlength corrected

Formula
☐ Use single formula for all wells

Current Formula:

Write formula and click in a cell to apply it

	1	2	3	4
A		STD1 x/0.472*0.5	STD1 x/0.467*0.5	
B		STD2 x/0.47*0.5	STD2 x/0.466*0.5	
C		STD3 x/0.468*0.5	STD3 x/0.464*0.5	
D		STD4 x/0.468*0.5	STD4 x/0.463*0.5	
E		STD5 x/0.467*0.5	STD5 x/0.461*0.5	
F		STD6 x/0.466*0.5	STD6 x/0.46*0.5	
G		SPL1 x/0.468*0.5	SPL1 x/0.461*0.5	
H		SPL2 x/0.47*0.5	SPL2 x/0.464*0.5	

Transformation

Data In: pathlength corrected

[Select multiple data sets...]

New Data Set Name: blanked A562

Formula
☑ Use single formula for all wells

Plate Formula: X-STD1

	1	2	3	4
A		STD1 X-STD1	STD1 X-STD1	
B		STD2 X-STD1	STD2 X-STD1	
C		STD3 X-STD1	STD3 X-STD1	
D		STD4 X-STD1	STD4 X-STD1	
E		STD5 X-STD1	STD5 X-STD1	
F		STD6 X-STD1	STD6 X-STD1	
G		SPL1 X-STD1	SPL1 X-STD1	
H		SPL2 X-STD1	SPL2 X-STD1	

4a. Create a transformation to calculate pathlength corrected ODs to a 0.5 mm equivalent, using the pathlengths given on the Take3 data sheet.

4b. Create a transformation to blank the pathlength corrected ODs, using the "0" STDs as the blanks.

4c. Create a curve step:
- "blanked A 562" is selected for the y-axis
- use the 2nd degree polynomial curve fit

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