

Pierce Protein Determination BCA assay (12/05;ARW)

1. Prepare standards curve

Lane	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
1 mg/ml BSA	0	.5	1	2	3	4	5	6	7	8	9	10
Reagent A	10	9.5	9	8	7	6	5	4	3	2	1	0

2. Prepare working reagent (Reagent C turns solution green)

For	2 Rows	3 Rows	Half Plate	5 Rows	6 Rows	7 Rows	Full Plate
Reagent A	2.75	4.13	5.50	6.88	8.25	9.63	11.00
Reagent B	2.64	3.96	5.28	6.60	7.92	9.24	10.56
Reagent C	0.11	0.17	0.22	0.28	0.33	0.39	0.44
Total	5.5	8.3	11.0	13.8	16.5	19.3	22.0

3. Prepare samples (dilute in Reagent A)

- a. For whole cell from cell culture dilute samples 1:5 and 1:10
 - b. For whole cell from tissue dilute samples 1:20 and 1:40
 - c. For nuclear enriched from tissue dilute samples 1:20 and 1:40
 - d. For membrane enriched from tissue dilute samples 1:5 and 1:20
 - e. For supernatants from tissue Nuc/Mb preps dilute samples 1:5 and 1:20
4. Pipette 10 λ of sample per well.
 5. Add 200 λ of working reagent per well, including standards.
 6. Mix gently by tapping or lowest vortexer setting.
 7. Cover with parafilm and incubate @ 37°C for 30'.
 - a. Turn on plate reader (requires ~5' warm-up).
 8. During incubation set up template if not stored.
 - a. Go to Template, select new template (fill out see example below)

Top is std curve, then samples top box (sample name) bottom box (dilution factor)

0	.05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
TC	TC	WC	WC	Nuc	Nuc	Mb	Mb				
5	10	20	40	20	40	5	20	...			

TC = Tissue culture WC = Whole cell tissue Nuc = nuclear enriched from tissue Mb = membrane enriched from tissue

9. Go to protocol, select reader setup.

- a. Select Single
 - b. Select λ 570
 - c. Select shake 3"
 - d. Hit OK
10. Remove plate from incubator and let cool to RT ~5' (std row should be light to dark purple).
 11. Place plate in machine, go to plate, select read plate, hit OK.
 12. Go to protocol, go to report, select customize, select mean conc., hit OK.
 13. Go to analysis, curve fit should be linear.
 14. When looking at readings click on wells above or below your standard curve to not be included in conc. average (well will be X'ed out).
 15. Go to print report you will have a table, graph, and your samples with conc.
 16. Throw away plate and turn off plate reader (Leave computer on).