Micropipette Experiment

Make Standards

1. Obtain 8 microcentrifuge vials and label B-I. Vial A is provided.
2. Add the appropriate volume of diluent (diionized water) to each vial.
	1. See chart below.
3. Add the correct amount of BSA (standard, *Vial A*) to each vial working from A to I.
	1. Make sure to vortex each vial after the BSA has been added and before moving 75 μL to the next vial.

| Vial | Volume of Diluent ( DI water) (μL) | Volume and Source of BSA (μL) | Final BSA Concentration (μg/mL) |
| --- | --- | --- | --- |
| A | 0 | 150 | 2000 |
| B | 75 | 75 from vial A | 1000 |
| C | 75 | 75 from vial B | 500 |
| D | 75 | 75 from vial C | 250 |
| E | 75 | 75 from vial D | 125 |
| F | 75 | 75 from vial E | 62.5 |
| G | 75 | 75 from vial F | 31.25 |
| H | 75 | 75 from vial G | 15.625 |
| I | 75 | 0 | 0 |



Make Sample Dilutions

1. Dilute your sample with diionized water according to the following chart. Labeling vials 1-4.

| **Dilution** | **Sample (μL)** | **Diluent (water) (μL)** |
| --- | --- | --- |
| 1 (1:2.5) | 30 | 45 |
| 2 (1:5) | 15 | 60 |
| 3 (1:10) | 7.5 | 67.5 |
| 4 (1:20) | 3.75 | 71.25 |

Make Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:
	1. (# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume of WR required
	2. You will do 2 replicates of each sample.
	3. You will have 9 standards and 4 unknowns as seen above.
	4. The volume of WR per sample is 200 μL.
	5. Make a couple hundred microliters extra.
		1. For example if you need 5.8 mL (5800 μL), make 6 mL (6000 μL) total WR.
2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). Use 15 mL centrifuge tube provided.
	1. For the above example:
		1. 6000 μL /50 = 120 μL of Reagent B and 6000 μL of Reagent A

Load Wells

1. Load plate wells with 25 μL of standards and sample dilutions using the grid below. The ‘ stands for the replicate. (A and A’ should come from the same vial)

| A | B | C | D | E | F | G | H | I |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| A’ | B’ | C’ | D’ | E’ | F’ | G’ | H’ | I’ |  |  |  |
| 4 | 4’ |  |  |  |  |  |  |  |  |  |  |
| 3 | 3’ |  |  |  |  |  |  |  |  |  |  |
| 2 | 2’ |  |  |  |  |  |  |  |  |  |  |
| 1 | 1’ |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |

1. Then add 200 μL of WR to each well and mix plate thoroughly on the belly dancer for 30 seconds.
2. Cover plate with parafilm and place in incubator for 30 min.
3. Cool plate to room temperature and measure absorbance at or near 562nm on a plate reader.
	1. Use *plate reader protocol*.
	2. Use “Hensley Protein Assay 562 Spr 14” in predefined methods.
	3. Save results to your folder in “Cell Biology Fall 2015.”