Homework #4 Calculations and Key

	1	2	3	4	5	6	7	8	9
Sample	%AS	cpm	- blank	μmoles (100ul)	μmoles/ml (% total)	A600	μg protein (10μl)	mg/ml	Sp. Act.
Blank		103							
Cytosol		10,482	10,379	0.104	1.04	0.765	14	1.40	0.74
A	20	319	216	0.002	0.02 (2)	0.107	1.44	0.14	0.15
B	35	1,497	1,394	0.014	0.14 (13)	0.254	4.23	0.42	0.33
C	50	8,708	8,605	0.086	0.86 (83)	0.409	7.18	0.72	1.20
D	65	10,165	10,062	0.101	1.01 (97)	0.549	9.85	0.99	1.02
E	80	9,748	9,645	0.096	0.96 (92)	0.689	12.5	1.25	0.77

- The percent saturated $(NH_4)_2SO_4$ (column 1) is calculate from the nomogram. (Note: mg added per ml is the same as grams per liter.)
- The cpm of binding activity in the blank (column 2) need to be subtracted from each of the values (column 3).
- Divide the net cpm (column 3) by the specific activity of the radioisotope (10^5 cpm/μmole) to give the μmoles of drug bound in the assay sample(column 4).
- Divide by 0.1 ml to get binding activity per unit volume (column 5) which is equal to the total amount of binding activity since sample volume is one ml. The percent of the total (ie, cytosol) represents the recovery.
- Convert the A_{600} values (column 6) to μg protein (column 7) using a standard curve generated from known amounts of protein (see next page for example).
- Divide the μg protein by the volume measured (10 μl) to get the protein concentration (column 8). (Note: μg/μl = mg/ml)
- The specific activity (binding activity per mg protein) is determined by dividing the column 5 by column 8.

The 50% sat. $(NH_4)_2SO_4$ gave the optimal enhancement of the specific activity. However, a better recovery was obtained at 65% sat. $(NH_4)_2SO_4$. One could further optimize the procedure by looking at smaller increments between 50-65% saturation. In addition, a 2-step procedure could be carried out where the proteins precipitated at 20% sat. $(NH_4)_2SO_4$ (or perhaps higher) were discarded before carrying out the 50% sat. $(NH_4)_2SO_4$ (or perhaps higher) precipitation.

The amount of protein is calculated by plotting the A_{600} of the protein standards against the amount of protein (see graph below). The amount of protein in an unknown standard is then determined from the plot (dashed lines). The values can also be used in linear regression analysis and the protein amounts calculated.