*Do not include the homework in your laboratory notebook. Turn in the assignment directly to your instructor.*

**Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. How would you prepare a 10 ml protein solution of 0.5 mg/ml starting from a stock with a concentration of 2 mg/ml? **(1 point)**
2. You are given 1 ml of a 100 mM stock solution of a new enzyme inhibitor. Describe and calculate how to prepare 500 µl of inhibitor diluted 1:100 dilution. Be specific including volumes. What is the new concentration? Also describe and calculate how to dilute 10 µl of the same inhibitor 1:1000. What is the new concentration of this dilution? **(1 point)**

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1. You have a 10 µg/ml solution of a pure protein called mitochondrial malate dehydrogenase (MDH). Perform a quick internet search to determine the mass of human mitochondrial MDH. What is the molecular weight of human mitochondrial MDH? What is the molarity of MDH in your solution using your mass in daltons? **(1 points)**
2. Explain what you would do to make a 10.0 ml of a 10 µM solution of caffeine starting from solid caffeine. The molecular weight of caffeine is 456 AMU. Be detailed in order and amount of additions. Is there something wrong with this result – describe any issue (the anwer is yes… you figure out what and why)? Without being very wasteful, what other means might you do to make up this solution? **(2 points)**
3. You are given the following: 0.5 M KH2PO4 , 0.5 M K2HPO4 , 0.5 M Tris base, 0.5 M Tris HCl and 3 M NaCl, and solutions of NaOH and HCl. Use the internet to find the molear mass for each compound. Calculate how you would prepare of each of the following (do not use a website – show all math):
   1. 10 ml of Buffer A (10 mM Tris-Cl pH 8.0 , and 50 mM NaCl)
   2. 10 ml of Buffer B (10 mM Tris-Cl pH 8.0 , 100 mM NaCl )
   3. 10 ml of Buffer C (10 mM Tris-Cl pH 8.0 , 500 mM NaCl)
   4. 50 ml of Buffer D (50 mM K+ Phosphate buffer, pH 8.0).
4. Describe what a 5X solution means? Why do we use the “5X” as an informal unit? **(2 points)**
5. A buffer used to denature proteins includes the following components:

* 25 mM Tris-HCl pH 8.2, 8 M Urea, 200 mM Glycine, 10 mM ß mercaptoethanol (ßME) and 250 mM NaCl.

In the laboratory you have a 1 M solution of Tris buffer at pH 7.0, a bottle of ßME at 14.3 M and solid urea, glycine and NaCl.

* Calculate how you would prepare this buffer. Provide a step by step description of how to make of the buffer with details including rinsing glasses, how you would measure each item, volumes of water added to beakers and so on… Use the back of this page for your answer. **(5 points)**

1. What reaction does MDH catalyze? Is there a “forward” direction to the enzyme?

9)What is the difference (molecular weight, genes, cellular location, metabolitic pathways) between human cytosolic MDH and human mitochondrial MDH. What is a mitochondrial transit peptide? Does this peptide remain after MDH is placed into the mitochondria? How does porcine MDH differ from human MDH?

10) What metabolic pathway(s) does cytosolic and mitochondrial MDH play a role in?