

Name _____

Biochemistry Lab II
Mid-term Exam
Due: Monday, April 15

Take-home, open book, open note, absolutely no assistance from humans (including chatting or asking questions online). You are free to use internet sources... just be sure of them.

Your exam consists of designing a plan for purifying and characterizing alcohol dehydrogenase from yeast (*Saccharomyces cerevisiae*) which has been over-expressed in *E. coli* cells. (Note: The gene for the yeast alcohol dehydrogenase has been put on a plasmid that *E. coli* can transcribe and translate. The enzyme is in *E. coli*. Do NOT start with yeast.)

In other words, you are provided with *E. coli* that is expressing yeast alcohol dehydrogenase. You don't have to figure out how to get the gene into the *E. coli*.

You should include:

1. Purification scheme with your reasoning behind each step. (This means step by step instructions for what you would do to purify the enzyme. You don't have to include exact volumes or concentrations.)
2. Where you can purchase the materials you need for your purification and characterization scheme, complete with catalog numbers. Hint: Most catalogs are online.
3. A proposed purification table (it doesn't have to be filled out). This would include your stages.
4. How you will assay the enzyme
5. How you propose to characterize the kinetics of this enzyme and why you chose that method. (This would include proposed concentrations for the substrates in the assays and what type of analysis you would plan to do and why.)
6. Any references you use. (It's fine to use sources, that's where most research starts, just give them credit.)

Good luck!