Names: Key

1. For each of the steps indicate where you expect succinate dehydrogenase to be. Talk through the reasoning behind your decision with your partner.

Step	Supernatant	Pellet
Centrifugation of crude liver homogenate at 1700 rpm	X	
Centrifugation of crude liver homogenate at 14000 rpm		X
Centrifugation of 28% ammonium sulfate solution at 30,000 g	X	
Centrifugation of 40% ammonium sulfate solution at 30,000 g		X

2. Does ammonium sulfate prefer to interact with biopolymer surface (protein) or water more strongly? Explain how you know.

Ammonium sulfate stabilizes protein structure and even causes 4° interactions that are not biologically relevant (i.e., pellet formation). It does not like to interact with protein biopolymer surface.

3. Determine the expected molecular mass of chicken (Gallus gallus) succinate dehydrogenase using the UniProtKB database.

77.931 kDa + 32.597 kDa + 16.435 kDa

4. The cellulose dialysis tubing used in this purification has a 12 kDa (12,000 g mole^{-1}) molecular weight cutoff. During the dialysis step, list all the components that you expect to exchange with the surrounding equilibration solution.

Hepes; Dodecyl-β-D-maltoside; ammonium sulfate; Thesit; sucrose.

5. During the dialysis step, list all the components that you expect to stay within the dialysis membrane.

Succinate hydrogenase proteins

6. What is the role of the 10 mM Hepes? Why is it required?

Hepes acts as a buffer. Since proteins have ionizable side chains in the amino acids, pH is important to maintaining the protonation state needed for molecular interactions that stabilize the protein structure

7. What is the role of the 3 g/L Thesit? Why is it required?

Thesit is a detergent which disolves the mitochondrial membranes. It will interact with the portion of the protein normally within the membrane and stabilize protein structure.