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Datum Sheet for Laboratory 7
Succinate Dehydrogenase Activity in Cauliflower Mitochondria

A. Measurement of Succinate Dehydrogenase activity in different fractions

1. In this experiment, you analyzed the succinate dehydrogenase activity in 3 different cellular fractions (nuclear, mitochondrial, and supernatant). Which fraction would you expect to have the highest succinate dehydrogenase activity and why? (In other words, what is your hypothesis for this experiment?)(1 point)

2. Raw Data: Record the absorbance for reading for each tube in the table below: (1 point)

	Tube							
Time	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
3								
6								
9								
12								
15								
18								
21								
24								
27								
30								

3. **Attach to this datum sheet a graph with the rate plots for tubes # 2 through # 5**, which you measured the activity of the crude homogenate (H), nuclear fraction (N), mitochondrial fraction (M), and supernatant fraction. Then Draw a best-fit line through the **linear portion** of each datum set in each assay. (2 points for the format of the graph).

4. Calculate the slope of the line based on your graph and include the calculation Rise/Run on the graph, record values below. (NO NEGATIVE VALUES remember we are looking for a change in absorbance!!) (2 points)

#2 Slope Crude _____ ΔA_{600}/min

#3 Slope Nuclear _____ ΔA_{600}/min

#4 Slope Mitochondrial _____ΔA_{600}/min

#5 Slope Post Mitochondrial Supernatant _____ΔA_{600}/min

5. Convert the slopes (from #3) to ΔA_{600}/min ml by dividing by the mL of enzyme used (0.5 mL) (2 points)

#2 Crude _____ ΔA_{600}/min mL

#3 Nuclear _____ ΔA_{600}/min mL

#4 Mitochondrial _____ΔA_{600}/min mL

#5 Post Mitochondrial Supernatant _____ΔA_{600}/min mL

6. Use the conversion factor for DCPIP (1 nmole/ 0.0215 A_{600} unit) to convert the activities (from #4) into in nmoles/min mL being sure to adjust by the volume of the reaction for each fraction (3 mL) (2 points)

#2 Crude _____ nmoles/min mL

#3 Nuclear _____ nmoles/min mL

#4 Mitochondrial _____ nmoles/min mL

#5 Post Mitochondrial Supernatant _____ nmoles/min mL

7. Fill in the following chart using the values you found for tubes # 2 through # 5 (3 points). Volumes From part A (your manual) activities from #5 and #6 on the previous page. (3 points)

Fraction	Volume (ml)	Activity (nmoles/min ml)	Total Activity (nmoles/min)
Homogenate			
Nuclear			
Mitochondrial			
Supernatant			

You can calculate the total SDH activity in each fraction by multiplying the volume of each fraction by its activity.

8. Which of the fractions appear to have the most SDH activity (nmoles/min ml)? (2 points) Explain.

9. Now calculate the percentage of the succinate dehydrogenase activity in homogenate which was recovered in each of the other fractions. That is, divide the total activity in the nuclear, mitochondrial, and supernatant fractions by the activity in the homogenate and multiply by 100% (1.5 points).

Fraction	Percent of SDH Activity Recovered
Nuclear	
Mitochondrial	
Supernatant	

10. Do your results support or refute your hypothesis from #A1? Explain (2 points)

B. Measurement of Succinate Dehydrogenase Activity when components of the mixture have been removed.

1. In this experiment, you analyzed the succinate dehydrogenase activity in when different components of the reaction mixture were omitted. Which component(s) would you expect to have the largest effect on succinate dehydrogenase activity and why? (In other words, what is your hypothesis for this experiment?)(1 points)

2. Attach to this datum sheet a graph with the rate plots for tubes # 6 through # 8 in which you measured the activity of the mitochondrial fraction in reaction mixtures from which the assay medium, the sodium azide, or the succinate was omitted. Include tube 4 for comparison. Draw a best fit line through the (**linear portion of each datum set 6-8)** (2 points).

3. Calculate the slope of each line on the graph and include your calculation on the graph for each set of data. Give the rate of each reaction based on the slope of the line from your graph in the linear portion of each line, as a ΔA_{600}/min. NO NEGATIVE VALUES (1.5 points)

   Slope Reaction #6 No Assay _____ ΔA_{600}/min

   Slope Reaction # 7 No Azide _____ ΔA_{600}/min

   Slope Reaction # 8 No Succinate _____ΔA_{600}/min

4. Adjust the slopes from C5 to ΔA_{600}/min mL or relative activity being sure to adjust by the volume of the reaction for each fraction. (1.5pts)

   Reaction # 6 Assay _____ ΔA_{600}/min mL

   Reaction # 7 No Azide _____ ΔA_{600}/min mL

   Reaction # 8 No Succinate _____ΔA_{600}/min mL

5. Now convert the activities from C6 into in nmoles/min mL being sure to adjust by the volume of the reaction for each fraction as in part B (1.5pts)

   Reaction # 6 Assay _____ nmoles/min mL

   Reaction # 7 No Azide _____ nmoles/min mL

   Reaction # 8 No Succinate _____ nmoles/min mL

6. Fill in the following chart using the values you found for tubes # 6 through # 8 comparing them to the mitochondrial fraction from part B Tube 4. Activities from #3 above in nmoles/min mL. Fill in the following chart (2 points).

Reaction Mixture	Component Omitted	Activity (nmoles/min ml)	Percentage
4	None		100%
6	Assay Medium		
7	Na Azide		
8	Succinate		

7. What do these results mean? Which component had the most effect? Which had the least effect? (1

point)

8. Do your results support or refute your hypothesis from #B1? Explain (1 point)