



## Procedure

{ See schematic table on next page }

1. Add 2.0 mL of yeast to a clean large (13 x 150 mm, from your drawer) test tube and heat it in a boiling water bath for 5 min. Allow it to cool, then place it in the warm water bath at 40°C. Label this tube as the boiled yeast tube (*e.g.*, boiled).
2. Add 2.0 mL of yeast to each of four additional clean test tubes and place them directly in the 40°C water bath (without boiling). Add 5 drops of 0.2% potassium cyanide (KCN) to **one** (only) of these test tubes. Label the tube containing KCN (*e.g.*, cyanide).
3. After the solutions have had 5 min to allow the temperature to equilibrate, add 1 drop of 0.1% methylene blue solution to each of the 5 test tubes (including the one heated in step 1). This should give each tube a distinctly blue color.
4. To the 5 test tubes add the following: add 2.0 mL of 5% sodium lactate solution to the test tube containing the boiled yeast; add 2.0 mL of 5% sodium lactate solution to the test tube containing KCN; add 2.0 mL of 5% sodium lactate solution to one test tube without KCN {label it lactate}; add 2.0 mL of 5% ethanol solution to another test tube (label it EtOH); finally add 2.0 mL of distilled water to the last test tube {label it blank}. Be sure to label the test tubes to avoid confusing their contents. Place a cork in each test tube and keep each tube in the warm water bath at about 40°C.
5. Make sure the contents are thoroughly mixed and light blue at the start, and keep the test tubes in the warm water bath at 40°C for 10 min. Note any changes in color of the solution. Record your observations for each tube on the Report Sheet.
6. After recording your observations for the color of each solution on the Report Sheet, take note of whether there is any color at the very top of the tube. Shake each tube for a few seconds, noting whether there is any change in color as you shake the tube. If the blue color reappears, what is causing this color to reappear?
7. Remove the cork from each test tube and shake the tube well. You may also try blowing air through the tube with a dropper by immersing an empty dropper in the solution and squeeze the dropper bulb. Keep squeezing the bulb as you pull the dropper out of the solution to avoid getting the solution in the dropper. If you get solution in the dropper, be sure to rinse it with water and remove all water from the dropper before blowing air into the next tube.
8. Answer all the questions on the Report Sheet.

### Schematic for Test Tubes

Tube # →	1 (Boiled)	2 (+ KCN)	3 (Lactate)	4 (EtOH)	5 (H <sub>2</sub> O blank)
Yeast Solution	Add 2.0 mL of warm yeast solution to each test tube at the start				
Boiling treatment	Heat yeast in boiling water bath for 10 min	Do Not Heat to Boiling			
Temperature Equilibration	Place all test tubes in a water bath at 40°C for 5 min to equilibrate				
Cyanide Solution	No KCN	5 drops KCN	No KCN	No KCN	No KCN
Methylene Blue	Add 1 drop of 0.1 % methylene blue solution to each test tube				
Substrate solution	2.0 mL of 5% Sodium Lactate	2.0 mL 5% Sodium Lactate	2.0 mL 5% Sodium Lactate	2.0 mL of 5% Ethanol	2.0 mL of deionized water
Observations	After mixing each tube thoroughly, take note of any changes in the color of each tube as a result of the reduction of Methylene Blue				

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Name \_\_\_\_\_

Section \_\_\_\_\_

## **Metabolism in Yeast**

Experiment # 12

Pre-Lab Exercise

1. How would you classify this enzyme, lactate dehydrogenase in terms of the enzyme classification scheme described in the text book, *i.e.*, oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase?
  
2. How would you classify the enzyme, alcohol dehydrogenase?
  
3. Methylene blue is a dye that is used in this experiment as an indicator for oxidation and reduction. What color is methylene blue in the oxidized state?

What color is methylene blue in the reduced state?

4. Boiling yeast may result in these organisms no longer being able to metabolize nutrients. Explain what may be destroyed as a result of boiling.



Name \_\_\_\_\_ Section \_\_\_\_\_

## Carbohydrate Metabolism in Yeast

Experiment #12

Data & Report Sheet

### Lactic Acid Dehydrogenase and Alcohol Dehydrogenase Reactions in Yeast

	Observations	
	After 10 min in water bath	After shaking in air
5% Na lactate + boiled yeast		
5% Na lactate + KCN		
5% Na lactate		
Blank, distilled water		
5% Ethanol		

#### Questions:

1. What accounts for the differences in your observations between the blank tube containing yeast with no substrate and the tube containing 5% sodium lactate or 5% ethanol. Why does sodium lactate or ethanol result in the loss of color in this tube?

