

The effects of catalase, like those of all enzymes, are influenced by the surrounding temperature. Temperature has an effect on both the structure of the catalase itself and the hydrogen bonds it is designed to cleave. As the temperature increases toward the optimum point, hydrogen bonds loosen, making it easier for catalase to act on hydrogen peroxide molecules. If the temperature increases beyond the optimum point, the enzyme denatures, and its structure is disrupted.

### Standard Preparation

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1. Aliquot 50mL samples of 1.5% peroxide substrate into three separate 125mL Erlenmeyer flasks. Label the flasks A, B, and C respectively.
2. Perform steps 3 – 6 independently of one another to ensure that each flask is used directly after reaching its designated temperature. Do not move on to the next flask until the entire procedure has been completed with its predecessor.
3. Place flask A on ice to decrease the temperature to 5 °C.
4. Flask B should be kept at a room temperature of 25 °C.
5. Place flask C on the [OHAUS Guardian 7000 Hotplate Stirrer](#). Connect the external probe to the back of the device and submerge the probe into the sample solution. The “Probe in Sample” icon will illuminate on the display. Heat the sample to a final temperature of 52 °C.
6. Once the flask that is being used reaches designated temperature, add 100µL of 1000mg/mL enzyme catalase solution to the sample being prepared.
7. Connect a spirometer to a computer and place the probe into the flask being tested. Use the spirometer’s stopper to seal the flask.
8. The stopped flask is then placed on the [OHAUS Guardian 7000 Hotplate Stirrer](#). Enable the SmartRate™ feature with a slow ramp rate to prevent splashing. The turtle icon will be seen on the display. Set the stir speed to 500 RPM to begin the stirring process.
9. Allow the reaction to occur for 200 seconds (The timer display of the [OHAUS Guardian 7000 Hotplate Stirrer](#) will count up to 3 minutes, 20 seconds). Ensure that the spirometer is turned on and collecting data throughout the entirety of the 200 seconds.
10. Using the spirometer’s measurements, calculate both the line of best fit and slope to represent the enzymatic rate of the reaction.
11. After completing this for all three flasks, compare your results to determine the ideal conditions for an enzymatic reaction.

### OHAUS Products Used Within This Procedure

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OHAUS Guardian 7000  
Hotplate Stirrer