Experimental Enzymology



322 BCH



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Mark Distribution and Dates of the Exam:

| | Marks | |
|---------------------------|-------------|----------|
| Conducting the experiment | 5 Marks | |
| | | |
| Report | 15 Marks | |
| Quiz | 10 Marks | |
| Midterm (30 Marks) | Practical | 15 Marks |
| | Theoretical | 10 Marks |
| Final (40 Marks) | Practical | 25 Marks |
| | Theoretical | 15 Marks |
| Total | 100 Marks | |

Date of midterm exam: 11/2/1439H -31/11/2017 Date of final exam: 17/3/1439 H - 5/12/2017

The Aim of This Course

- How Enzyme work and the specify of the enzyme
- To understand how to characterize Enzyme



Course Outline:

Title of the Experiments

- **1** Some Factors Affecting Polyphenol Oxidase Activity
- 2 Methods of Enzyme Assay
- 3 Purification of Acid Phosphatase from Wheat Germ
- 4 The Effect of Incubation Time on the Rate of an Enzyme Catalyzed Reaction
- 5 The Effects of Temperature on the Rate of an Enzyme Catalyzed Reaction
- 6 The Effects of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction
- 7 The Effects of pH on the Rate of an Enzyme Catalyzed Reaction
- 8 The Effects of Substrate Concentration on the Rate of an Enzyme Catalyzed Reaction
- 9 The Inhibition of Acid Phosphatase by Inorganic Phosphate
- 10 Modification of Active Site Cysteine of Lactate Dehydrogenase

Things to know before starting a lab



Basic information



Understand













How to write a scientific report?



The laboratory reports should contain the following sections:

- L) First Page : Course number, Title, Name
- 2) Objectives
- 3) Brief Introduction [Theoretical background information around 15 20 lines]
- 4) Materials and Methods
- 5) Results [Tables, Graphs and/or Calculations]
- 6) Discussion : In this section you are required to describe of what happened in the experiment, explain your results and make conclusions by comparing your results to expected values (calculated or from the literature). Even if you obtained unexpected results, the discussion section is the section to justify or explain the reasons why you have obtained such results.

7) Questions

Safety in the Lab:



- You must wear a lab coat and hand gloves.
- Open toed shoes must not be worn because they cannot protect you against chemical spills.
- Long hair should be tied back to avoid any interference with the experiment.
- In case of acid or base contact with your skin, wash it with large amount of clean, cold water and inform the instructor immediately.
- Do not handle broken glassware with your bare hands.
- Do not eat, drink, or chew gum in the laboratory.
- Do not depart from the lab leaving an experiment unattended. If you need to leave the lab you must inform your instructor before leaving the lab.
- You must wash your hands with soap after finishing the experiment.







- Hormones
- Enzymes
- Antibody
- Structural proteins







- What are Enzymes and its function?
- **Enzymes** are molecules catalyze(speed up chemical reactions) the biochemical reactions that occur in cells.
- Chemically, enzymes are like any catalyst and are not consumed in chemical reactions,

• Not all enzymes are proteins / not all proteins are enzymes!!!



- There are 3 terms for an enzymatic reaction to happen:
- 1. Presence of enzyme
- 2. Presence of substrate
- 3. Availability of appropriate conditions (37 C, pH 6.7)
- →If any of these terms are absent there will be no enzymatic reaction.
- \rightarrow Give some examples of enzymes in the body

Important Terms:



- Catalysis: the change in rate of a chemical reaction due to the participation of a substance called a catalyst = Enzymes.
- *Enzyme:* a biological molecule that increase the rates of chemical reactions (mostly reversible) without being consumed.
- **Substrate**: a molecule upon which an enzyme acts on.
- *E-S complex*: an intermediate formed when the substrate molecule binds to the active site of the enzyme.
- *Product*: a substance produced as a result of the reactions.
- Active site: is the region of an enzyme where substrate molecules bind and undergo a chemical reaction.

Studying Enzymes

Because hundreds of reactions are simultaneously carried out in the living cell, it becomes difficult to study a single reaction in an intact living cell.

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However, it is possible to extract enzymes from cells and thus study enzyme catalyzed reactions in a test tube.



How to asses enzymatic reaction in the lab?



Notes in practical work:



- In any experiment you should know the aim and what you are doing while working
- You must know why and what are the importance of the chemicals that you are adding during the experiment
- You should be accurate
- Instruments:
- Spectrophotometer
- Pipettes
- Micropipette
- Waterbath

Useful websites

<u>http://www.ebi.ac.uk/enzymeportal/</u>

http://bkm-react.tu-bs.de/

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Animation:

 <u>http://highered.mheducation.com/sites/0072495855/st</u> <u>udent_viewo/chapter2/animation_how_enzymes_wo</u> <u>rk.html</u>

Homework

• Get information on polyphenol oxidase