

Methods of Enzyme Assay

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Introduction:

- All enzyme assays measure either the **consumption of substrate or production of product over time.**
- **Different enzymes require different estimation methods depending on the type of reaction catalyzed, the nature of S and P or coenzyme.**

Methods of quantitatively following enzyme reaction:

1-Spectrophotometric methods.

2-Fluorescence methods: using a fluorometer . E.g. NAD⁺ and NADP⁺ do not fluoresce in their oxidized forms, but the reduced form have a blue fluorescence reduction reaction.

3- Sampling methods : by withdrawing samples at intervals and estimating the substrate or product by chemical methods. It is for inorganic phosphate. It can be used for phosphatases, phosphorylases, nucleotides and all enzymes involving ATP or ADP including some kinases and synthetases enzymes.

4- Manometric methods: Use manometer ,suitable and accurate methods for following reactions in which one of the component is a gas. e.g. Oxidases (O₂uptake), Decarboxylases (CO₂output)

5- Eelectrode Methods: reactions which involve the production of acids. In this method pH meter is used to measure change in H⁺ conc. During enzyme reactions. (i.e. measure change in pH as the reaction proceeds).

6- Polarimetric Method: use polarimeter. For isomerases that convert one isomer to another.

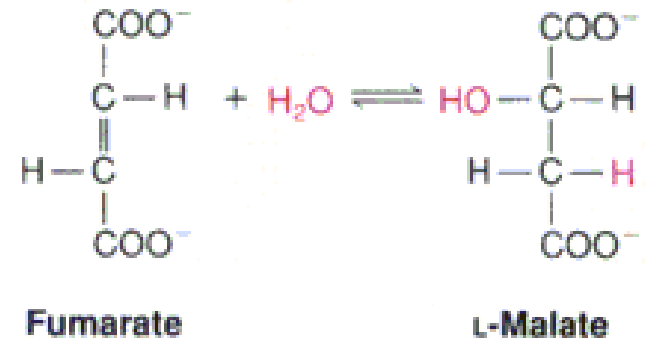


When the Spectrophotometric methods can be used?

1) Cases in which product absorbs but not the substrate.

e.g. Fumarate hydratase.(catalyze the addition of groups to double bonds)

Fumarate \longrightarrow Malate



2) The Co-enzyme Nicotinamide Adenine

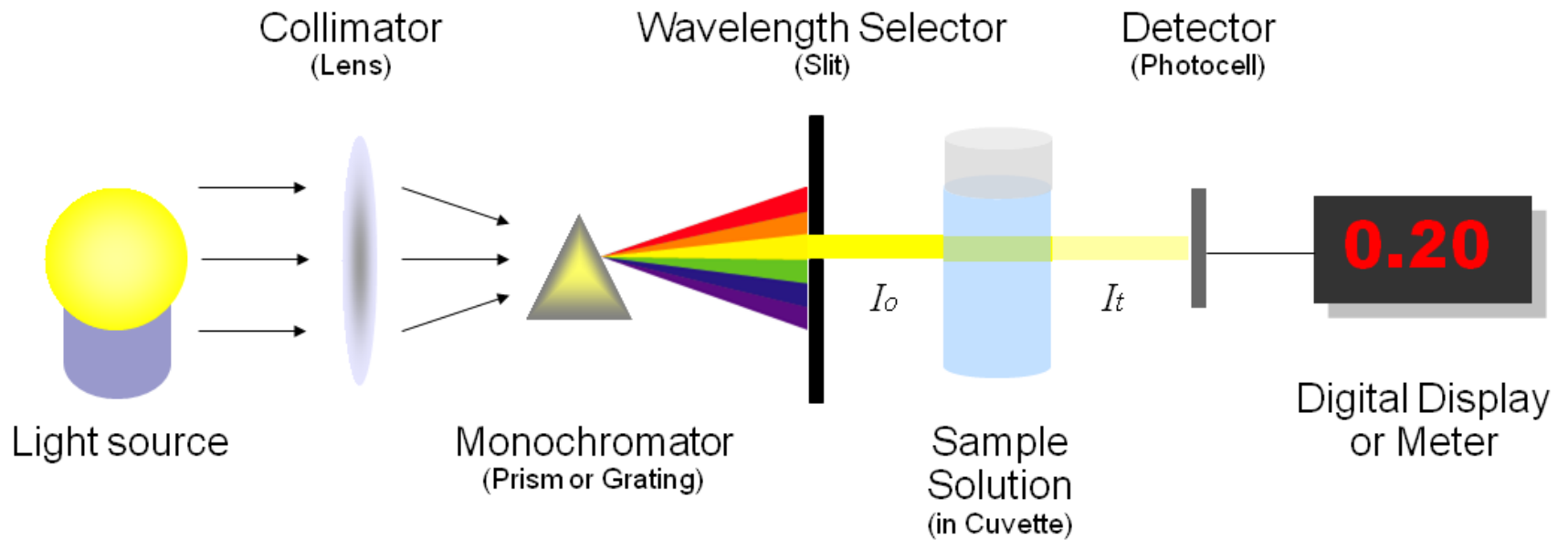
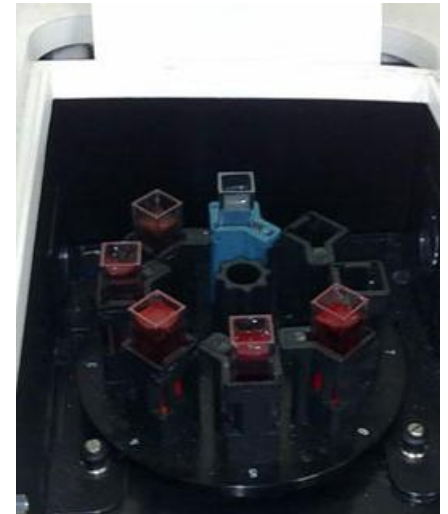
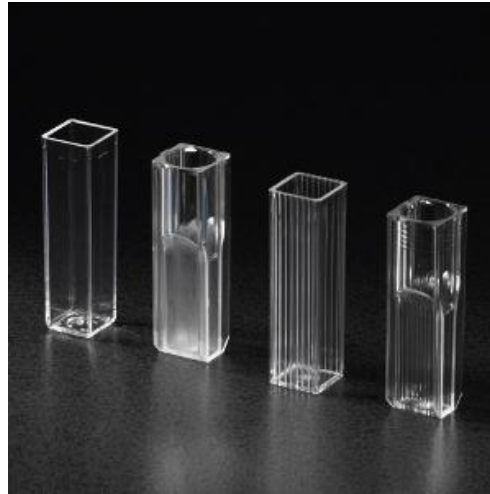
Dinucleotide/ Nicotinamide Adenine Dinucleotide Phosphate

NAD/ NADP have an absorption band at 340 nm in the reduced state

NAD⁺ \longrightarrow NADH

NADP⁺ \longrightarrow NADPH

Oxidized form \longrightarrow Reduced form



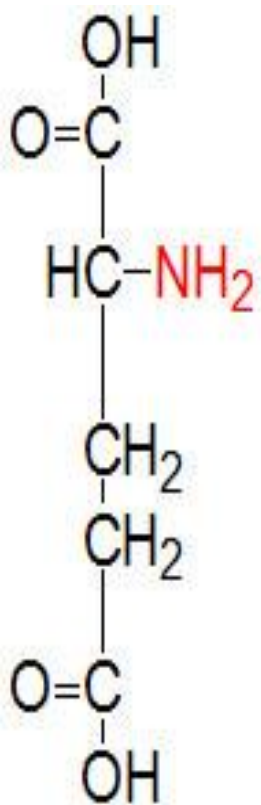
Enzyme assays can be:

- **Continuous assay** , where the assay gives a continuous reading of activity.
- **Discontinuous assay**, where the samples are taken, the reaction stopped then the concentration of substrates/products determined.

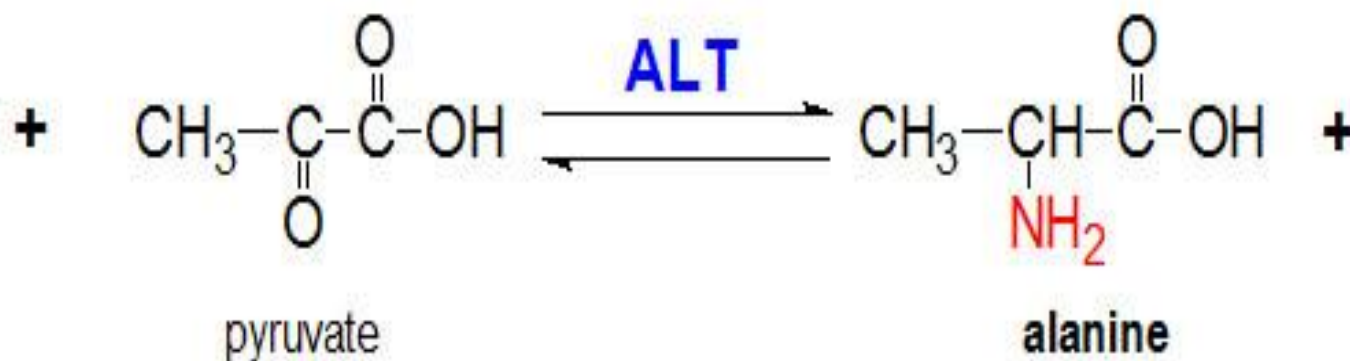
Alanine transaminase

An enzyme that catalyzes a type of reaction between an amino acid and α -keto acid.

Specifically, this reaction (**transamination**) involves removing the amino group from the amino acid, leaving behind an α -keto acid, and transferring it to the reactant α -keto acid and converting it into an amino acid.



glutamate



pyruvate

alanine

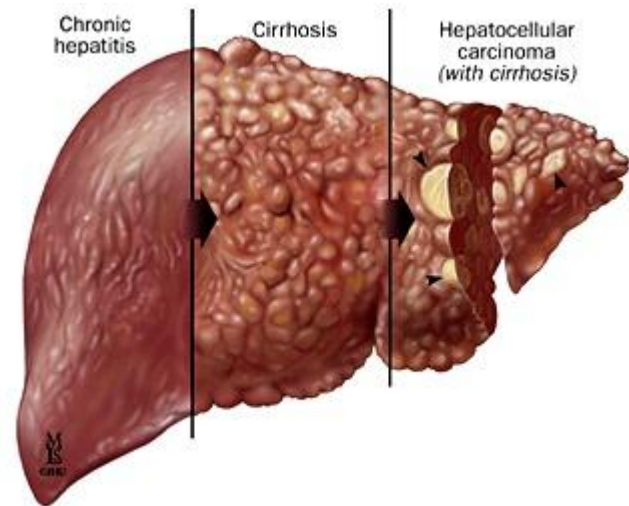
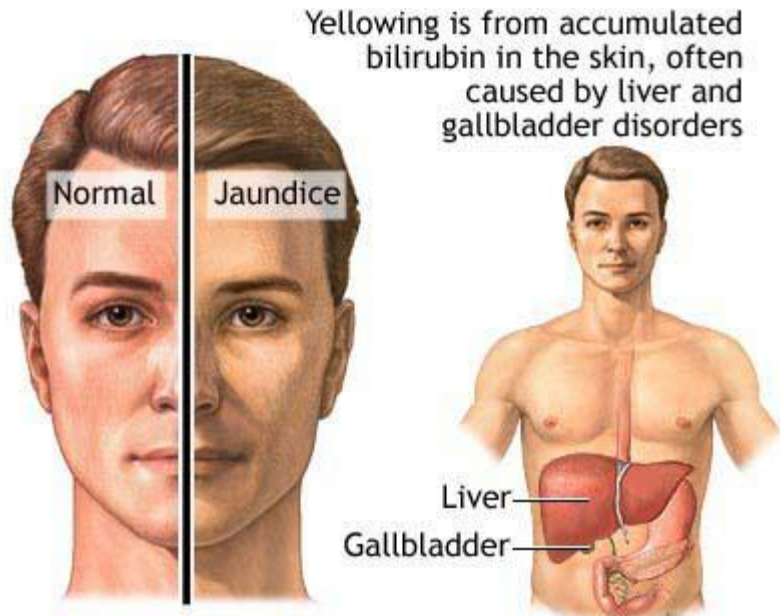
α -ketoglutarate

The enzymes are **important** in the **production of various amino acids**, and measuring the concentration of various transaminases in the blood is important in the diagnosing and tracking many diseases.

Alanine transaminase or ALT is a transaminase enzyme. It is also called: Serum glutamic pyruvic transaminase (**SGPT**) or Alanine aminotransferase (**ALAT**). ***ALT is found in serum (at low level) but is most commonly associated with the liver.***



ALT found mainly in liver cells: thus , an elevated level ALT is a sensitive index of acute hepatocellular injury. Elevated serum ALT(SGPT) level are found in hepatitis, cirrhosis , and obstructive jaundice. Levels of ALT (SGPT) are only slightly elevated in patient following a myocardial infraction.



Objectives:

Study the Continuous Assay method by determining the enzymes activity for :

- 1. Alanine transaminase**
- 2. Lactate dehydrogenase**

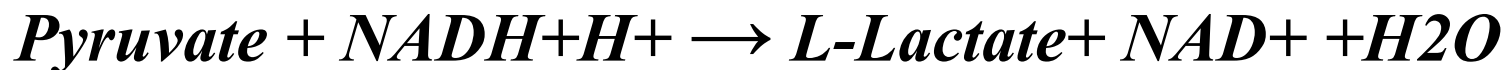
1-Alanine Transaminase Assay

Principle:

It catalyzes the transfer of an amino group from alanine to α -ketoglutarate, to form pyruvate and glutamate under controlled condition (37°C) and pH 7.4 ± 0.05



The pyruvate formed in the reaction is reduced to L-Lactate by Lactate dehydrogenase (LDH) with the Oxidation NADH. Measure the absorbance of NADH/NAD⁺ at **340nm**



Material

A- Chemicals

ALT (SGPT) reagent:

- 100 mmol/L Tris (PH =7.5 , \pm 0.05)
- 350 mmol/L L-alanine
- 15 mmol/L 2-Oxoglutarate with preservative
- 0.25mmol/L NADH
- \geq 5000 U/L LDH with filler and stabilizer

Serum Sample

B- Instruments:

- Quartz cuvette(invisible region)
- Spectrophotometer (340nm)
- Stop watch

1-Alanine Transaminase Assay

Method:



T1

Pipette **3ml** of the ALT reagent

Pre-warm the tubes at **37 for 3 min**

Pipette **0.2ml /200µl** of serum sample

Mix , and allow 60 seconds for temperature equilibration

Read the absorbance at **340nm** every minute for 3 minute /use(H₂O) as blank

Results:

Test Tube	Absorbance at 340nm		$\Delta A/\text{min}$
T1	A1		$((A1-A2)+(A2-A3))/3$
	A2		
	A3		

ALT Activity (U/L) = $\Delta A/\text{min} \times 1768$

ALT Activity (U/L) = U/L

NORMAL RANG OF ALT: up to 32 U/L female
up to 42 U/L male

Unit definition

: $\Delta A/\text{min}$ = measured the rate of change in absorbance per min

(U/L) = the amount of enzyme that will reduce one micromole of NADH per min per liter of sample at specific temperature.

2-Lactate Dehydrogenase Assay

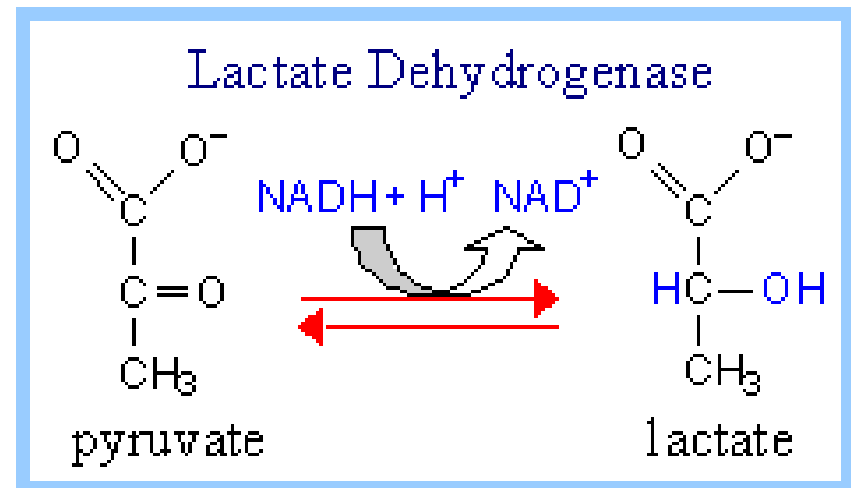
Use the co-enzyme in measure the activity of Lactate dehydrogenase:

An enzyme that catalyzes the **conversion of lactate to pyruvate**. (LDH)

This is an important step in energy production in cells.

Many different types of cells in the body contain this enzyme. Some of the organs relatively rich in LDH are the **heart, kidney, liver, and muscle**. **It is responsible for converting muscle lactic acid into pyruvic acid, an essential step in producing cellular energy.**

Lactic acid dehydrogenase (LDH) is present in almost all of the tissues in the body and becomes **elevated in response to cell damage**. LDH levels are measured from a sample of blood taken from a vein. Generally, the upper limit of normal for adults is in the range of 200 units/liter. **Elevated level** of LDH in serum are found in myocardial infraction, liver diseases, renal diseases, certain forms of anemia, malignant diseases and progressive muscle dystrophy.



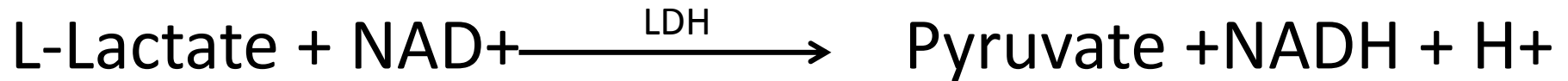
Low LDH can be seen in malnutrition, hypoglycemia, adrenal exhaustion, or low tissue or organ activity.



2-Lactate Dehydrogenase Assay

Principle:

LDH catalysis the following reaction:



The rate of NADH formation is indicated by **increase** the **absorbance** at 340nm and it is **directly proportional to serum LDH activity**.

If:

NADH is **product** : **increase** the absorbance /min

NADH is **reactant**: **decrease** the absorbance /min

Material:

A- Chemicals

LDH reagent:

- 80 mmol /L Buffer (PH =8.6 , \pm 0.05)
- 70 mmol /L Lithium L-Lactate
- 5.5 mM NAD⁺
- Non reactive stabilizer with preservative.

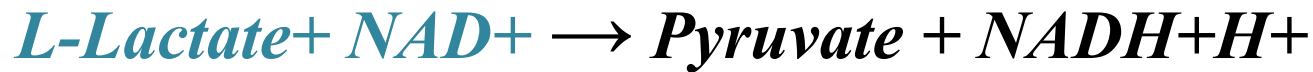
Serum Sample.

B- Instruments:

- Quartz cuvette (invisible region)
- Spectrophotometer (340nm)
- Stop watch

2-Lactate Dehydrogenase Assay

Method: **LDH**



T1

Pipette **3ml** of the LDH reagent

Pre-warm the tubes at **37 for 3 min**

Pipette **0.4 ml/400µl** of serum sample

Mix , and allow 60 seconds for temperature equilibration

Read the absorbance at **340nm every minute for 3 minute** /use(H₂O) as blank

Results:

Test Tube	Absorbance		$\Delta A/\text{min}$
T1	A1		$((A3-A2)+(A2-A1))/3$
	A2		
	A3		

LDH Activity (U/L) = $\Delta A/\text{min}$ x 4984

LDH Activity (U/L) = U/L

**NORMAL RANG OF LDH: (103- 277) U/L female
(80-285) U/L male**

Thank You

