Isoenzymes:

Isoenzymes (also known as isozymes) were first described by Hunter and Markert (1957). Isoenzymes mean a group of enzymes catalyze the same reaction but they differ in chemical structure i.e. they differ in amino acid sequence which changes the electric charge of enzyme, thus they differ in physical, immunological, electrophoretic properties, as well as display different kinetic parameters (K_M and V_{max}) and different regulatory properties.

Differences in structure of isoenzymes do not affect the active sites.

Subunits of isoenzyme are encoded by different genes, but enzymes that their subunits are encoded by different alleles of the same gene, they are described as allozymes.

To identify isozymes, a crude protein extract is made by grinding animal or plant tissue with an extraction buffer, and the components of extract are separated according to their charge by different ways e.g. Gel electrophoresis.

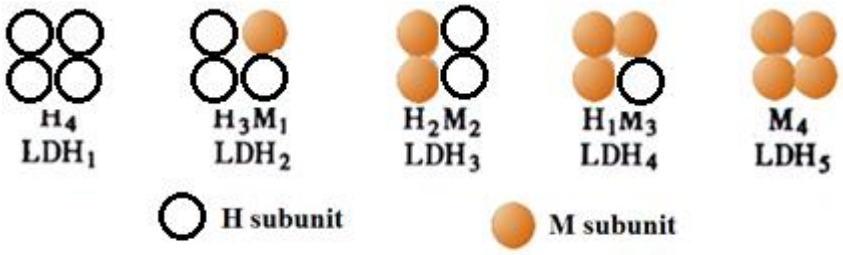
Many isoenzymes contain different subunits in various combinations,

such as Lactate dehydrogenase (LDH) and Creatine phosphokinases (CK).Frequently, different organs contain characteristic proportions of different isoenzymes. these isoenzymes are of value in the diagnosis of muscle, heart and brain diseases.

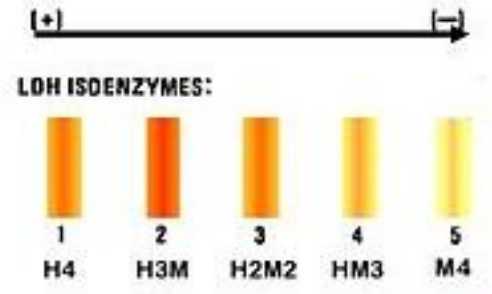
-Lactate dehydrogenase (LDH):

LDH is a tetramer composed of two different subunits H and M, the H form for heart-specific and the M form for muscle-specific, combines in one of five combinations as shown in below:

Type	Composition
LDH_1	HHHH
LDH_2	HHHM
LDH_3	HHMM
LDH_4	HMMM
LDH_5	MMMM



Isoenzymes of lactate dehydrogenase



The differences between 5 isozyme when use Electrophoresis

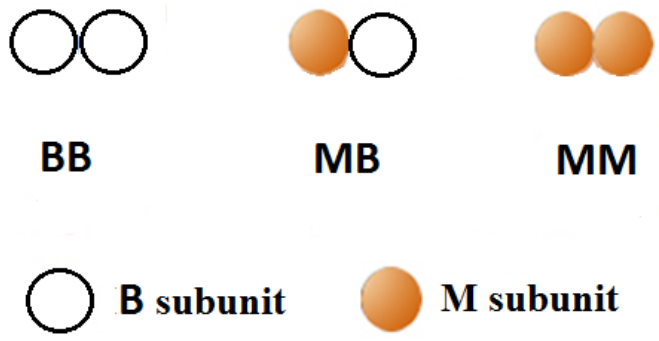
-Creatine kinase CK (Creatine phosphokinase CPK):

CK is a dimer composed of two different sub units M and B, the M form for muscle-specific and the B form for brain-specific, combines in one of three combinations as shown in below:

CK_1 CK-BB

CK_2 CK-MB

CK_3 CK-MM



isoenzymes of creatine kinase

The existence of isoenzymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage.

An example of an isoenzyme is glucokinase, a variant of hexokinase which is not inhibited by glucose 6-phosphate. Its different regulatory features and lower affinity for glucose (compared to other hexokinases), allows it to serve different functions in cells of specific organs, such as increase insulin secretion by the beta cells of the pancreas, or initiation of glycogen synthesis by liver cells. Both of these processes must only occur when glucose is abundant.

Units of enzyme activity:

The quantity or concentration of an enzyme can be expressed in molar amounts, as with any other chemical, or in enzyme activity.

In order to express the enzyme activity in unit terms, it is necessary to ensure that the assay procedure used is measuring the true initial velocity and it is proportional to the enzyme concentration. This can be valuable for assessing the effects of physiological and pharmaceutical factors on cell or tissues, monitoring the purification of enzymes and comparing the activities of different enzymes, data of a certain enzyme from different laboratories, different sources or different substrates.

Enzyme activity is measured in many units as follow:

1- International units (IU):

One I.U. is defined as the amount of enzyme that converts one micromole of substrate to product per minute. (1 IU) = 1 μmol min^{-1}).

2-Specific activity:

It is defined as the number of international units (I.U.) per milligram of protein. (μmol $min^{-1}mg^{-1}$). When the mass of protein in an assay is known. Specific activity gives a measurement of enzyme purity in the mixture .The value becomes larger as an enzyme preparation becomes more pure.

3-Katal (kat):

It is a new unit for measuring enzyme activity. One katal (kat) indicates the amount of enzyme that converts one mole of substrate to product per second. (1 katal = 1 mol s^{-1}).

1 IU = (1/1000000)/60 kat

1 IU =16.67×10^{-9} kat.

1 IU = 16.67 nkat.

Katal is the SI unit, but this is an excessively large unit. A more practical and commonly used value is international unit.

4- Catalytic constant (Kcat) or Turnover number (TN):

It is number of substrate molecules converted into product by a single catalytic site (or one enzyme molecule if it has one active site) per time unit (minute or second) at when the enzyme is saturated with substrate. Catalytic constant is measured by following equation:

$$\text{Catalytic constant (Kcat)} = \frac{Vmax}{[ET]}$$

V_{max} = maximum velocity

[ET] = total enzyme concentration

Kcat measures number of catalytic cycles that each active site undergoes per time unit. High Kcat means fast reaction and low Kcat means slow reaction. Most enzymes have Kcat between 10^2 and 10^6 per second.

For example, carbonic anhydrase has a catalytic constant of 800,000 to 1000,000 s^{-1}, which means that each carbonic anhydrase molecule can produce up to 1000,000 molecules of product per second.

Catalytic Constants of Some Enzymes

Enzyme	k_{cat} (s^{-1})
Staphylococcal nuclease	95
Cytidine deaminase	299
Triose phosphate isomerase	4300
Cyclophilin	13,000
Ketosteroid isomerase	66,000
Carbonic anhydrase	1,000,000

5-Catalytic efficiency:

Enzymes are important for a variety of reasons, most significantly because they are involved in many chemical reactions that help us to maintain our daily lives. Increasing the reaction rate of a chemical reaction allows the reaction to become more efficient, and hence more products are generated at a faster rate. This is known as catalytic efficiency of enzymes and it is a best value to represent the enzyme's overall ability to convert substrate to product. Catalytic efficiency is measured by following equation:

$$\text{Catalytic efficiency} = \frac{\text{Kcat}}{\text{Km}}$$

Kcat = Turnover number

Km = Michaelis constant

It is a useful indicator for comparison of activity of an enzyme for different substrates.

Kcat, Km, and Kxat/Km for some enzymes and substrates

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1	4.1×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8