Swemed DIAGNOSTICS

LDH – KINETIC KIT

PRINCIPLE:

LDH Catalyzes the following Reaction

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+}$$

CONTENTS:

1. Reagent R1 : Ready – To – Use
2. Reagent R2 : Ready – To – Use

PRECAUTIONS:

Avoid Contamination of Ready - To - Use Reagents. Always use fresh pipette tips. Keep always the cap tightly closed.

REAGENT PREPARATION:

The above Reagents R1 & R2 are Ready – To – Use and are stable until expiry dates mentioned on the label. Mix 4 parts of R1 with 1 part of R2. The combined reagent is stable for 5 days at 2 – 8^{0} C.

COLLECTION AND HANDLING OF SPECIMEN:

1. Serum with any visible hemolysis can not be used because of the contamination of this sample with large amount of LDH released from the Erythrocytes.

2. Serum should be separated from the clot promptly.

3. Samples should be assayed soon after collection. LDH in serum is reported to be stable for two to three days at room temperature.

4. The liver LDH is particularly labile and is destroyed if frozen and thawed.
   Equilibriate reagent tubes at 37^{0} C before addition of sample.

ASSAY PARAMETER:

Mode : Kinetic.
Wave Length : 340nm.
Cuvette : 1cm path length.
Reaction Slope : Decreasing.
Temperature : 37^{0} C.
Blank : Distill Water.
Reagents Volume: 1000 ul.
Sample Volume : 25 ul.
Delay time : 60 sec.
Reaction time : 180 sec.
Number of reading: 3.
Factor : 8199
Linearity : 1000 Iu/L.
Uints : Iu/L.

MANUAL ASSAY:

Pipette into cuvettes	Macro	Semi-Micro
Reagent(R1+R2)	800+200 ul	400+100 ul
Sample	25 ul	12.5ul

Mix & take the first reading at 340 nm after 60 Sec. and take three additional readings at 60 Sec. intervals. Calculate mean absorbance change per minute (A/min)

CALCULATIONS:

One International Unit (IU) is defined a the amount of enzyme that catalyzes the transformation of one micromole of NADH per minute under specific temperature.

IU /L = Abs./min x 8199

EXPECTED VALUES (at 37^{0} C):

Male 80 – 285 U/L
Female 100 – 230 U/L

LINEARITY : 1000 U/L

REFERENCES:

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