

LIQUID L-LACTATE TRINDER

ENZYMATIC COLORIMETRIC DETERMINATION IN BIOLOGICAL FLUIDS

Kit: 1 x 50 ml

Code LAT8840

SUMMARY

L-Lactate is an intermediary in the carbohydrate metabolism. It is derived from white skeletal muscle, brain, skin, renal medulla and erythrocytes.

The most important diseases, connected to lactic acidosis, are present in two different clinical settings:

- type A (hypoxic), associated with the decrease of tissue oxygenation,
- type B (metabolic), associated with diabetes mellitus, neoplasia and liver diseases; it is also associated with drugs/toxins and inborn metabolism defects.

PRINCIPLE

The L-Lactate is changed in pyruvate and hydrogen peroxide by L-LOX (lactate oxidase).

The hydrogen peroxide reacts in presence of peroxidase (POD) with chromogen precursors giving a purple compound.

The intensity of color is proportional to the concentration of L-Lactate in the tested sample.

REAGENTS

Components of the kit:

- | | |
|--|---------------------|
| | Code LAT8840 |
| * REAGENT 1 (liquid, ready to use) | 1 x 42 ml |
| * REAGENT 2 (liquid, ready to use) | 1 x 8 ml |
| Good buffer > 20 mmol/L pH 7,2 | |
| Chromogen precursors > 1 mmol/L | |
| L-Lactate Oxidase ≥ 200 U/L | |
| Peroxidase ≥ 900 U/L | |
| Excipients, stabilizer, preservatives. | |
| * REAGENT 3 (standard low, liquid, ready to use) | 1 x 2 ml |
| (values mentioned on the bottle) | |
| * REAGENT 4 (standard elevated, liquid, ready to use) | 1 x 2 ml |
| (values mentioned on the bottle) | |

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

AUXILIARY REAGENTS (Not supplied with this kit)

To assure proper test performance, we suggest following kits:

- SUBSTRATE Elevated Control (Iyo) 6 x 2 mL Cod. SCE3005
- SUBSTRATE Low Control (Iyo) 6 x 2 mL Cod. SCL3006

that contain a few Substrates at two different levels (see the inserts).

PREPARATION OF THE WORKING REAGENT

Add to one vial *Reagent 2 a small quantity of *Reagent 1. Mix gently until complete solution, then transfer the solution to the biggest plastic bottle. Mix gently, until complete solution. Please avoid foaming.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagent reach the working temperature before use.

Mix kindly before use.

Close immediately after handling. The Reagents have to be used correctly, to avoid contamination.

Incompetent handling will release us from any responsibility.

STABILITY: 2 months at 2-8°C or 2 weeks at 15-25°C in the dark.

Till 4 months freezed at -20°C. FREEZE only ONE TIME.

DO NOT REPEAT FREEZING.

SAMPLE

- Plasma: (Lithium heparin iodoacetate, Fluoride EDTA, fluoride oxalate, NaF).
On recommend to keep samples on ICE and to separate the plasma from blood without delay.
- CSF: use without modification.

PROCEDURE

- Wavelength: 550 nm (530-570)
- Pathlength: 1 cm
- Reading: against Blank Reagent
- Temperature: 37°C
- Method: end point
- Reaction: 10 minutes
- Linearity: up to 120 mg/dL
- Sample/Reagent: 1/100

Let reagents reach the working temperature before using.

Pipette in a test tube or cuvette so labelled:

R/B: Reagent Blank, S: Sample, St: Standard;

	R/B	ST	S
Working reagent	1000 µl	1000 µl	1000 µl

Wait that the tubes reach 37°C then add:

Distilled water	10 µl	----	----
*Reagent 3 Std Low and *Reagent 4 Std Elev.	----	10 µl	----
Sample	----	----	10 µl

Mix and read the absorbance of standard (Ast) and sample (As) after 10 minutes of incubation at 37°C against Blank Reagent.

CALCULATION

(AS / Ast) x C* = Concentration of L-Lactate.

C* is the value of the Standard Low and Elevated in mg/dL.

REFERENCE VALUES

L-Lactate

Plasma (from Fasting Venous Blood)
3 - 12 mg/dL (0,33-1,33 mmol/L)

CFS 10 - 22 mg/dL (1,1 - 2,4 mmol/L)

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The L-Lactate Reagent is linear up to 120 mg/dL (13,3 mmol/L).

For concentrations ≥ 120 mg/dL, dilute the sample 1:2 with saline solution, repeat the determ. and multiply the result x 2.

Sensitivity: The minimum detectable is 1,5 mg/dL.

Within-run Precision:

	Mean (mg/dL) ± 2s	CV %
Serum 1	8,1 ± 0,21	1,3
Serum 2	37,3 ± 0,63	0,84

Run-to-run (Day-to-day) Precision:

	Mean (mg/dL) ± 2s	CV %
Serum 1	8,2 ± 0,4	2,04
Serum 2	37,5 ± 0,8	1,06

Interferences: See References point 2.

Correlation: A group of 18 sera from 4 to 58 mg/dL was assayed by this procedure and using a similar commercially available L-Lactate Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,002x - 0,10$

Correlation coefficient $r = 0,9988$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than 120 mg/dL dilute the sample 1:2 with saline solution, repeat the determination and multiply the result by 2.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of L-Lactate (see References 2.).
5. **PAY ATTENTION!**
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).

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