BIOLABO
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L.D.H. (LDH-P) SFBC Modified Method

Reagent for quantitative determination of Lactate Dehydrogenase activity [EC 1.1.1.27] in human serum or plasma.

REF 92111	**R1** 1 x 150 mL	**R2** 10 x 15 mL
REF 92011	**R1** 1 x 60 mL	**R2** 20 x 3 mL

TECHNICAL SUPPORT AND ORDERS
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Latest revision : www.biolabo.fr

CE

IVD

Made in France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (automated method). It allows the quantification of global activity of LDH enzyme in human serum.

I GENERALITES (1) (4) (5)

Lactate dehydrogenase (LDH) activity is present in all cells of the body. Enzymes levels are particularly high, compared with those in serum, in liver, heart, kidney, skeletal muscles and erythrocytes. In addition to their higher enzyme activity, many of these tissues show different isoenzyme composition (separable by electrophoresis).

PRINCIPLE (1)

UV Kinetic Method (SFBC):

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

The decrease in absorbance due to the conversion of NADH to NAD^+, directly proportional to LDH activity in the specimen, is measured at 340 nm.

REAGENTS COMPOSITION

R1	**LDH (LDH) P**	Substrate-Buffer
Buffer Tris pH 7.2	80	mmol/L
Pyruvate	1.6	mmol/L
Preservative		

R2	**LDH (LDH) P**	Coenzyme
NADH	≥ 0.20	mmol/L
NaCl	200	mmol/L

These Products are not classified as dangerous according to CLP Regulation (EC) 1272/2008

I SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

REF 92011: Add promptly 3 mL of R1 into vial R2.

REF 92111: Add promptly 15 mL of R1 into vial R2.

Recap vial and mix gently until complete dissolution.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagents are stable when stored and used as described in the insert:

Unopened,

- Until expiry date stated on the label.

Once opened and reconstituted:

- Working reagent is stable for 60 days when free from contamination.
- Discard any reagent if cloudy or if absorbance at 340 nm is < 1.100.

SPECIMEN COLLECTION AND HANDLING (2) (4)

Unhemolysed serum. Do not use heparinised plasma.

ALT is stable in serum or plasma for:

- 24 hours at room temperature.
- 7 days at 2-8°C.

LIMITS (3) (4)

LDH contained in reagent allows, during pre-incubation step, the reduction of endogenous pyruvate which would positively interfere. Elevated ALT level may involve NADH depletion during pre-incubation stage, which may lead to under-estimated results. In case of lipemic or It's recommended to check these specimens diluted (1 + 4) in saline solution.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 EXATROL-P Level II
- External quality control program

It is recommended to control in the following cases:

- At least once a run
- At least once within 24 hours
- When changing vial of reagent
- After maintenance operations on the instrument

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (4)

Adult LDH activity: at 37° C: 200-400 IU/L (SFBC method)

Note: Values for children are all the higher as children is young.

Each laboratory establishes its own normal ranges for the population it serves.

PERFORMANCES

On Kenza 240TX, 37°C, 340 nm.

Linearity Range: between 32 and 1500 IU/L

Detection limit: approx. 14 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	117	339	1085
S.D. IU/L	3.0	6.4	21.8
C.V. %	2.5	1.9	2.0

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	116	339	1099
S.D. IU/L	2.6	6.2	24.2
C.V. %	2.3	1.8	2.2

Analytical Sensitivity: approx.. 0. 0147 ΔAbs/min for 100 IU/L.

Comparison studies with commercially available reagent on human specimens (n=101) from 33 to 1500 IU/L:

y = 0.9998 x – 0.8942 r = 0.9996

Interferences:

Turbidity	No interference up to 0.349 abs
Total bilirubin	Negative interference from 333.5 µmol/L
Direct bilirubin	No interference up to 423.4 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1014 mg/dL
Haemoglobin	Positive interference from 19 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 1 month

Calibration Stability: 1 month

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

CALIBRATION

- REF 95015 Multicalibrator traceable to ERM-AD453

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

PROCEDURE

Manual method :

Let stand reagents and specimens at room temperature

Pipette into 1 cm pathlength thermostated cuvette:	
Reagent	1000 µL
Bring to 37° C then add:	
Calibrator, control, Specimen	20 µL

Mix. Start a timer.

After 30 seconds, record initial absorbance at 340 nm (or 334 nm)
Record the absorbance again after 1 minute and 2 minutes.

Calculate absorbance change per minute (ΔAbs/min.).

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other applications proposal are available on request.

CALCULATION

With seric multicalibrator:

$$\text{LDH Activity (IU/L)} = \frac{(\Delta\text{Abs/min) Assay}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Activity}$$

With theoretical factor:

Activity (U/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR} \times 1000}{6.3 \times \text{VE} \times \text{P}}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
6.3 = Molar extinction coefficient for NADH at 340nm
P = Pathlength (cm).

Example, with Manual Procedure,

(Pathlength 1 cm, 37°C, 340 nm):

IU/L = (ΔAbs/min) x 8095

$$\mu\text{Kat/L} = \frac{\text{UI/L}}{60}$$

REFERENCES

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(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 648-651*
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(4) *VASSAULT A., MAIRE I., SEBILLE L. AND BOZON D., Recommandations pour la mesure de la concentration catalytique de la lactate déshydrogénase dans le sérum humain à +30° C, Ann. Biol. Clin. (1982), 40, p.123-128*
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