BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

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
REF 92511	**R1** 10 x 50 mL	**R2** 10 x 50 mL

TECHNICAL SUPPORT AND ORDERS
Tel : (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC

CLINICAL SIGNIFICANCE (1) (6)

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).

For patients having an acute myocardial infarction, serum total LDH values become elevated at 12 to 18 h after the onset of symptoms, peak at 48 to 72 h, and return to below the upper reference limit after 6 to 10 days.

Elevation of LDH activity is also observed in renal disease, liver disease, anemia, pulmonary embolism, cancers and muscle lesions or diseases.

PRINCIPLE (4) (5)

Henry and al. method (according to SFBC recommendations).
Reaction scheme is as follows:

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

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

Vial R1 **SUBSTRATE-BUFFER**

Buffer Tris pH 7.2	80	mmol/L
Pyruvate	1.6	mmol/L
Preservative		

Vial R2 **COENZYME**

NADH	≥ 0.20	mmol/L
NaCl	200	mmol/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove aluminium cap.

REF 92511: Add promptly the contents of vial R2 (Coenzyme) into vial R1 (Substrate-Buffer).

REF 92011: Add promptly 3 mL of vial R1 (Buffer) into the contents of vial R2 (Coenzyme).

REF 92111: Add promptly 15 mL of vial R1 (Buffer) into the contents of vial R2 (Coenzyme).

Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C.

- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 30 days when free from contamination.
- Discard any reagent if cloudy or if absorbance measured at 340 nm is < 1.100.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING.(2) (4)

Unhemolysed serum or heparinised plasma, promptly removed from clot. Avoid fluoride, iodoacetate, sodium citrate, EDTA as anticoagulant.

Lithium heparinate does not disturb LDH activity measurements.

LDH activity is stable in specimen for 48 h from 4° C to 20° C.

Freezing will destroy liver isoenzymes and lead to a loss of activity of 10 to 20 % after 48 hours.

INTERFERENCES (3) (4)

Triglycerides:	No significant interference up to 1000 mg/dL
Bilirubin:	No known interference
Hemolysis:	Positive interference due to LDH activity released by erythrocytes.

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

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Use the theoretical calibration factor (§ **CALCULATION**)
- Or REF 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
- or a multiparametric calibrator traceable to a reference method or material

QUALITY CONTROL

- BIOLABO EXATROL-N (Level I) REF 95010
- BIOLABO EXATROL-P (Level II) REF 95011
- Other control sera assayed referring to the same method.
- 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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.
5. 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)	**at 30° C: 140-280 IU/L**

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

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

PERFORMANCE CHARACTERISTICS

Within-run N = 20	Normal level	High level
Mean IU/L	221	1464
S.D. (IU/L)	2.15	8.52
C.V. (%)	0.97	0.58

Beetwen run N = 42	Normal level	High level
Mean IU/L	340	922
S.D. IU/L	12.26	31.6
C.V. %:	3.61	3.43

Detection limit: approximately 20 IU/L.

Sensitivity for 810 IU/L: approximately 0.100 Abs/min at 340 nm.

Comparison studies with commercially available reagent:

y = 1.05 x - 25.41 r = 0.9992

LINEARITY

The assay is linear up to 1500 IU/L (25.5 µKat/L).

If ΔAbs/min > 0.185, dilute specimen (1 + 4) with saline solution and reassay taking into account the dilution factor. Linearity depends on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into 1 cm pathlength thermostated cuvette:	
Reagent	1 mL
Bring to 37° C then add:	
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.).	

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With theoretical factor:

IU/L = (ΔAbs/min) x 8095 at 340 nm

$$\mu kat/L = \frac{IU/L}{60}$$

With seric multicalibrator:

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

REFERENCES

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(3)
(4) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-372 à 3-377*
(5) *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*
(6) *HENRY R.J.and Al., Am. J . Clin. Path. (1974), 61, p.108*
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Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with