LDH SCE mod. liquiUV

Lactate Dehydrogenase (EC 1.1.1.27)

Package Sizes

REF	12214	16 x 5 ml	Complete M-Test Kit
	12014	10 x 10 ml	Complete Test Kit
	12024	8 x 50 ml	Complete Test Kit

IVD

Method [1]

"Modified method" based on the recommendations of the SCE (Scandinavian Committee on Enzymes).

Principle

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

Contents

	12214	12014	12024
REF			
BUF	16 x 4 ml	10 x 8 ml	8 x 40 ml
SUB	1 x 16 ml	2 x 10 ml	8 x 10 ml
BUF	**Buffer/Substrate**		
	TRIS buffer (pH 7.35)		62.5 mmol/l
	Pyruvate		1.5 mmol/l
	Sodium azide		0.095 %
SUB	**Substrate**		
	NADH		0.75 mmol/l
	Sodium azide		0.095 %

Reagent Preparation

Procedure 1 with reagent start

The reagents are ready for use.

The reagents are stable, even after opening, up to the stated expiry date when stored at 2...8°C. BUF must be kept light protected. Contamination of the reagents must be avoided!

Procedure 2 with sample start

REF **12024**: Pour the entire contents of one bottle SUB into one bottle BUF, mix thoroughly.

REF **12214**: Pipette 1 ml from bottle SUB into one bottle BUF, mix thoroughly.

REF **12014**: Pipette 2 ml from bottle SUB into one bottle BUF, mix thoroughly.

The working reagent is stable for 3 weeks at 2...8°C and 3 days at 15...25°C. The working reagent must be kept light protected.

Specimen

Serum, heparinised or EDTA plasma.

Avoid hemolysis!

Loss of activity within 3 days 8% at + 4°C, 2% at 15...25°C.

Assay

Wavelength: Hg 334 nm, 340 nm, Hg 365 nm
Optical path: 1 cm
Temperature: 25°C, 30°C or 37°C
Measurement: against air (decreasing absorbance)

Warm the reagents and the cuvettes to the desired temperature. Temperature must be kept constant (± 0.5°C) for the duration of the test.

Procedure 1*

Pipette into cuvettes	25°C, 30°C	37°C
Sample	20 µl	10 µl
BUF	1000 µl	1000 µl
Mix, incubate for 1 - 5 min. at 25°C, 30°C or 37°C.		
SUB	250 µl	
Mix, read the absorbance after 1 minute and at the same time start the stop watch. Read the absorbance again exactly after 1, 2 and 3 minutes.		

Procedure 2*

Pipette into cuvettes	25°C, 30°C	37°C
Sample	20 µl	10 µl
Working reagent	1000 µl	1000 µl
Mix, read the absorbance after 1 minute and at the same time start the stop watch. Read the absorbance again exactly after 1, 2 and 3 minutes.		

* Semi-micro method; for macro methods double the volumes.

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Calculation

Using the absorbance readings calculate the mean absorbance change per minute (ΔA/min).

Calculate the LDH activity in the sample by multiplying ΔA/min using the following factors:

Procedure 1

	Hg 334 nm	340 nm	Hg 365 nm
U/l (25°C, 30°C) = Δ A/min. x	10275	10080	18675
U/l (37°C) = ΔA/min. x	20390	20000	37060

Procedure 2

	Hg 334 nm	340 nm	Hg 365 nm
U/l (25°C, 30°C) = Δ A/min. x	8250	8095	15000
U/l (37°C) = ΔA/min. x	16345	16030	29705

If control results are outside the allowable ranges, the calculation factor should be checked with a suitable calibrator material and adjusted using correction factors.

Conversion factor of the traditional units (U/l) in SI-units (kat/l):

1 U/l = 16.67×10^{-3} µkat/l

1 µkat/l = 60 U/l

Factor to convert results to the new IFCC recommended method:

U/l (LDH SCE) x 0.4796 = U/l (LDH IFCC).

Performance Characteristics

Linearity

If the absorbance change per minute (ΔA/min.) exceeds 0.150 at Hg 334 nm, 340 nm or 0.070 at Hg 365 nm dilute 0.1 ml of the sample with 0.9 ml physiological saline (0.9%) and repeat the assay using this dilution. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/en-ldhuv.pdf

www.human-de.com/data/gb/vr/en-ldhuv.pdf

Reference Values [2, 3]

Temperature	25°C	30°C	37°C	IFCC[4]
Adults [U/l]	120-240	160-320	225-450	
Men [U/l]				< 243
Women [U/l]				< 244
Children [U/l] (up to 12 months)	up to 500			

Quality Control

All control sera with LDH values determined by this method can be employed.

We recommend to use our animal serum based HumaTrol or our human serum based SERODOS quality control sera.

Automation

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

Note

BUF and SUB contain sodium azide (0.095%). Do not swallow. Avoid contact with skin and mucous membranes.

References

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