DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Liquid Reagents – ready to use

LDH-L

IFCC

2 Reagents

Diagnostic reagent for quantitative in vitro determination of lactate dehydrogenase (LDH) in human serum or plasma on photometric systems

REF	Kit Size	Content
D03300B	**1 x 1.25 mL**	1 x 1 L R1 + 1 x 0.25 L R2
D00662	**5 x 100 mL**	4 x 100 mL R1 + 1 x 100 mL R2
D00663	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
D00664	**5 x 25 mL**	4 x 25 mL R1 + 1 x 25 mL R2
D00665	**5 x 10 mL**	4 x 10 mL R1 + 1 x 10 mL R2
D89911	**5 x 50 mL**	4 x 50 mL R1 + 2 x 25 mL R2
D0431917	**5 x 62.5 mL**	4 x 62.5mL R1 + 1 x 62.5mL R2
DA0835	**5 x 50 mL**	5 x 40 mL R1 + 5 x 10 mL R2
DT1035	**4 x 62.5 mL**	4 x 50 mL R1 + 4 x 12.5mL R2
DK0733	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
DE1835	**2 x 62.5 mL**	2 x 50 mL R1 + 2 x12.5 mL R2

Additionally offered:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98481SV	1 x 5 mL	Control abnormal	Diacon P

TEST PARAMETERS

Method:	UV, kinetic, increasing reaction, IFCC
Wavelength:	340 nm, Hg 365 nm, Hg 334 nm
Temperature:	37 °C
Sample:	Serum, heparin plasma or EDTA plasma
Linearity:	up to 1200 U/L
Sensitivity:	The lower limit of detection is 5 U/L

SUMMARY [1,2]

Lactate dehydrogenase (LDH) is an enzyme, consisting of five different isoenzymes which catalyse the interconversion of L-lactate and pyruvate. LDH is present in the cytoplasm of all human tissues with higher concentrations in liver, heart and skeletal muscle, and lower values in erythrocytes, pancreas, kidney and stomach. Increased LDH activities are found in a variety of pathological conditions such as myocardial infarction, cancer, diseases of liver, blood or muscle. However, because of the lack of organ specificity, determination of its isoenzymes or other enzymes such as alkaline phosphatase or GPT (ALT) / GOT (AST) is necessary for differential diagnosis.

TEST PRINCIPLE

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
N-Methyl-D-Glucamine, pH 9.40	420 mmol/L
L-Lactate	65 mmol/L
Reagent 2:	
NAD+	50 mmol/L

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: Reagent 2 must be protected from light!
Close immediately after use
Avoid contamination
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability up to the indicated expiration date

Sample Start (working reagent):
Stability: at 2 – 8 °C 12 hours
at 15 – 25°C 2 hours
Protect from light!

SAMPLE STABILITY AND STORAGE [4]

Stability at 4 – 8 °C 6 weeks
at 20 – 25 °C 4 days
Discard contaminated specimens.

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into a cuvette	Blank	Sample/Cal
Sample	-	20 µL
Dist. water	20 µL	-
Reagent 1	1000 µL	1000 µL
Mix. Incubate for approximately 1- 5 min. Then add		
Reagent 2	250 µL	
Mix. Read initial absorbance after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 minutes Determine ΔA/min. = [ΔA/min sample] – [ΔA/min blank] during the linear part of the assay.		

Sample Start

Pipette into a cuvette	Blank	Sample/Cal
Sample	-	20 µL
Dist. water	20 µL	-
Working Reagent	1000 µL	1000 µL
Mix. Read initial absorbance after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. = [ΔA/min sample] – [ΔA/min blank] during the linear part of the assay.		

CALCULATION

With factor: (light path 1 cm)
LDH [U/L] = ΔA/min x Factor
Factors (37°C):
Substrate Start

Factor	at 340 nm	10080
	at 334 nm	10275
	at 365 nm	18675

Sample Start

Factor	at 340 nm	8095
	at 334 nm	8250
	at 365 nm	15000

With calibrator:

$$\text{LDH [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{activity calibrator [U/L]}$$

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

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REFERENCE RANGE *

	Female		Male	
	U/L	µkat/L		µkat/L
Adults [6]	< 247	< 4.12	< 248	< 4.14
Children [7]				
1 – 30 days	145 – 765	2.42 – 12.8	125 – 735	2.09 – 12.3
31 days - 1 year	190 – 420	3 17 – 7.01	170 – 450	2.84 – 7.52
1 – 3 years	165 – 395	2.76 – 6.60	155 – 345	2.59 – 5.76
4 – 6 years	135 – 345	2.25 – 5 76	155 – 345	2.59 – 4.76
7 – 9 years	140 – 280	2.34 – 4.68	145 – 300	2.42 – 5.01
10 – 12 years	120 – 260	2.00 – 4.34	120 – 325	2.00 – 5.43
13 – 15 years	100 – 275	1.67 – 4.59	120 – 290	2.00 – 4.84
16 – 18 years	105 – 230	1.75 – 3.84	105 – 235	1.75 – 3.92

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automated systems the test is suitable for the determination of LDH activities up to 1200 U/L.
In case of a manual procedure, the test is suitable for LDH activities which correspond to a maximal ΔA/min of 0.15 at 340 and 334 nm or 0.08 at 365 nm.
If these values are exceeded the sample should be diluted 1+10 with NaCl solution (9 g/l) and results multiplied by 11.

SENSITIVITY / LIMIT OF DETECTION

The lower limit of detection is 5 U/L

PRECISION

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	178	2.00	1.12
Sample 2	187	2.12	1.14
Sample 3	566	2.27	0.40

Inter-assay n = 25	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	170	1.62	0.95
Sample 2	176	2.48	1.41
Sample 3	566	3.61	0.64

SPECIFICITY / INTERFERENCES

No interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Triglycerides	2000 mg/dL
Hemoglobin	50 mg/dL

METHOD COMPARISON

A comparison between Dialab LDH-L (y) and the IFCC reference reagent (x) using 51 samples gave the following results: y = 0.949 x + 8.451 U/L; r = 0.998
A comparison with a commercially available test with 51 samples gave following results:
y = 0.992 x + 10.72 U/L; r = 0.997.

CALIBRATION

The use of an LDH Calibrator is optional.
We recommend the Dialab multi calibration serum **Diacal Auto**. This method has been standardized against the original IFCC formulation.

QUALITY CONTROL

All control sera with LDH values determined by this method can be used.
We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. In very rare cases, samples of patients with gammopathy might give false results [8].
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
3. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

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