

LDH FS*

DGKC 1970

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

Order Information

Cat. No.	Kit size					
1 4201 99 10 021	R1	5 x	20 mL	+ R2	1 x	25 mL
1 4201 99 10 026	R1	5 x	80 mL	+ R2	1 x	100 mL
1 4201 99 10 023	R1	1 x	800 mL	+ R2	1 x	200 mL
1 4201 99 10 704	R1	8 x	50 mL	+ R2	8 x	12.5 mL
1 4201 99 10 917	R1	8 x	60 mL	+ R2	8 x	15 mL
1 4201 99 90 305	R1	10 x	12 mL	+ R2	2 x	20 mL

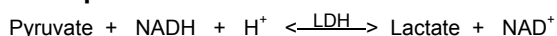
Summary [1,2]

Lactate dehydrogenase (LDH) is an enzyme, consisting of five different isoenzymes which catalyze 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 ALAT/ASAT is necessary for differential diagnosis.

Method

Optimized test according to German Society of Clinical Chemistry (DGKC) [3].

Principle



Reagents

Components and Concentrations

R1:	Phosphate buffer	pH 7.5	64 mmol/L
	Pyruvate		0.80 mmol/L
R2:	Good's buffer	pH 9.6	
	NADH		1.0 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and contamination is avoided. Do not freeze the reagents!

Reagent 2 must be protected from light.

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give false results [7].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. 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.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

Mix 4 parts of R1 + 1 part of R2

(eg. 20 mL R1 + 5 mL R2) = monoreagent

Stability:	5 days	at	2 – 8 °C
	8 hours	at	15 – 25 °C

The monoreagent must be protected from light.

Materials required but not provided

NaCl solution 9 g/L

General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Stability [4]:

4 days	at	20 – 25 °C
6 weeks	at	4 – 8 °C

Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	25 °C/30 °C/37 °C
Measurement	Against air

Substrate Start

Temperature	25 °C/30 °C	37 °C
Sample/Calibrator	20 µL	10 µL
Reagent 1	1000 µL	1000 µL
Mix, incubate for approx. 1 – 5 min., then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.		

Sample Start

Temperature	25 °C/30 °C	37 °C
Sample/Calibrator	20 µL	10 µL
Mono-reagent	1000 µL	1000 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.		

Calculation

With factor

From absorbance readings calculate $\Delta A/\text{min}$ and multiply by the corresponding factor from table below:

$\Delta A/\text{min} \times \text{factor} = \text{LDH activity [U/L]}$

Substrate Start	25 °C/30 °C	37 °C
340 nm	10080	20000
334 nm	10275	20390
365 nm	18675	37060

Sample Start	25 °C/30 °C	37 °C
340 nm	8095	16030
334 nm	8250	16345
365 nm	15000	29705

With calibrator

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

Conversion factor

$$\text{LDH [U/L]} \times 0.0167 = \text{LDH [\mu kat/L]}$$

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the molar extinction coefficient. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

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 maximum of $\Delta A/\text{min}$ 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.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 2,000 mg/dL triglycerides. Hemolysis interferes because LDH is released by erythrocytes. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 5 U/L.

Precision (at 25 °C)

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	142	5.50	3.86
Sample 2	245	4.95	2.01
Sample 3	497	8.39	1.69

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	144	3.09	2.13
Sample 2	248	4.53	1.82
Sample 3	492	6.23	1.26

Method Comparison

A comparison of DiaSys LDH FS (y) with a commercially available test (x) using 78 samples gave following results:

$$y = 1.03 x + 2.13 \text{ U/L}; r = 0.999.$$

Reference Range [6]



	25 °C	30 °C	37 °C	Unit
Adults:	< 240	< 346	< 480	[U/L]
	< 4	< 5.77	< 8	[μkat/L]

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

Literature

1. Thomas L. Clinical laboratory diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft;1998.p.89–94.
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3. Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. Z Klin Chem Klin Biochem 1972;10:182-92.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 36-7.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
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7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

Manufacturer

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