DiaSystem Scandinavia AB

Diagnostic reagent for quantitative in vitro determination of lactate in plasma and CSF on photometric systems

Order Information

Cat. No.	Kit size						
01 00029 70 04 0125	R1	5 x	20 mL	+	R2	1 x	25 mL
CDT-ALT	R1	3x	30 mL	+	R2	2x	11,3 mL

Summary [1,2]

Lactate is the final product of the anaerobic glycolysis and serves as indicator for the oxgen status in cellular tissues. Increased lactate levels in blood occur in anoxia due to shock, congestive heart failure, intoxication and thiamine deficiency. Therefore, lactate is measured in intensive care medicine. As metabolic variable for the capability of the muscles lactate determination is used in evaluation of the training status in athletes.

Method

Enzymatic UV test with lactate dehydrogenase (LDH).

Principle

L-Lactate + NAD^+ < LDH > Pyruvate + NADH + H^+

In the presence of NAD, lactate is converted by the lactate dehydrogenase. This procedure releases NADH which is measured at 340 nm. The absorbance of the produced NADH is proportional to the lactate concentration in the sample.

Reagents

Components and Concentrations

R1:	Buffer	pH 9.0	500 mmol/L
	LDH		≥ 25 kU/L
R2:	Nad		20 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, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Reagent 1: Danger. H315 Causes skin irritation. H318 Causes serious eye damage. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a poison center or doctor/physician.
2. Reagent 1 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [6].
5. 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.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

without Pyridoxalphosphate

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent
The stability of the mono-reagent is 14 days at 2 – 8 °C. Do not use icteric or hemolytic samples with sample start!

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Plasma and CSF (no serum)
Stability in plasma [3]:

8 hours	at	20 - 25 °C
14 days	at	2 - 8 °C

Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Substrate Start

	Reagent blank	Sample or calibrator
Sample or calibrator	-	15 µL
Dist. water	15 µL	-
Reagent 1	1000 µL	1000 µL
Mix and incubate 5 min. at 37 °C. Read absorbance A1 then add:		
Reagent 2	250 µL	250 µL
Mix and incubate 5 min. at 37 °C. Read absorbance A2 within 30 min.		

ΔA = (A2 – A1) sample/calibrator

Sample Start

(Do not use icteric or hemolytic samples)

	Reagent blank	Sample or calibrator
Sample or calibrator	-	10 µL
Dist. water	10 µL	-
Mono-reagent	1000 µL	1000 µL
Mix and incubate 5 min. at 37 °C. Read absorbance A within 30 min.		

Calculation

With factor

$$\text{Lactate [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal}} \times \text{Conc. Cal [mg/dL]}$$

With factor

From absorbance readings calculate ΔA and multiply by the corresponding factor from table below:

ΔA x factor = Lactate concentration [mg/dL]

	Substrate start	Sample start
340 nm	120.6	144.4

Conversion factor

Lactate [mg/dL] x 0.1109 = Lactate [mmol/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSystem UniCal CC calibrator is recommended. This method has been standardized against the original IFCC formulation. For internal quality control, Diasystem UniLab N and UniLab P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine lactate concentrations up to 120 mg/dL (13.3 mmol/L). When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2..

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated and unconjugated bilirubin up to 60 mg/dL, lipemia up to 2000 mg/dL triglycerides, hemoglobin up to 1000 mg/dL, dopamine up to 10 mg/L, L-dopamine up to 20 mg/L, methyldopamine up to 10 mg/L and glycolic acid up to 1200 mg/L. For further information on interfering substances refer to Young DS [4].

Sensitivity/Limit of Detection

The lower limit of detection is 1 mg/dL (0.1 mmol/L).

Precision

Intra-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	11,9	0,26	2,22
Sample 2	19,0	0,31	1,62
Sample 3	26,5	0,31	1,15

Inter-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	12,0	0,23	1,91
Sample 2	19,0	0,28	1,45
Sample 3	26,7	0,31	1,16

Method Comparison

With Pyridoxal-5-phosphate

A comparison of DiaSystem Lactate (y) with a commercially available assay (x) using 117 samples gave following results:
y = 0.984 x - 0.742 mg/dL; r = 0.999

Reference Range [5]

Plasma:

Venous	4.5 – 19.8 mg/dL (0.5 – 2.2 mmol/L)
Arterial	4.5 – 14.4 mg/dL (0.5 – 1.6 mmol/L)

CSF:

Adults	10 – 22 mg/dL (1.1 – 2.4 mmol/L)
Newborn	10 – 60 mg/dL (1.1 – 6.7 mmol/L)
3 – 10 days	10 – 40 mg/dL (1.1 – 4.4 mmol/L)
> 10 days	10 – 25 mg/dL (1.1 – 2.8 mmol/L)

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

Literature

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4. 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.
5. Section I – General Clinical Tests In: Tietz NW, editor. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia: Saunders; 1995. p. 382-3.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

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