LDH

DGKC method for determination of lactate dehydrogenase (LDH) activity in serum and plasma

CE IVD

ORDER INFORMATION

REF	Kit size
GA4546 00	5x40 + 1x20 ml
GA4547 00	10x40 + 2x20 ml
KL4546 00	2x50 + 2x5 ml
BK4546 00	2x(60+6 ml)

INDICATION

As LDH has an ubiquitarian nature, many conditions can cause an increase in the activity of this enyme. Alterations in LDH activity may be present in heart, liver, kidney, muscle, and hemolytic diseases.

METHOD PRINCIPLE

Optimized method according to "Deutsche Gesellschaft für klinische Chemie" (DGKC)[3].
LDH catalizes the following reaction:

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

Decrease of the absorbance value at 340 nm, due to the NADH oxidation in NAD^+, is directly proportional to the enzyme activity.

COMPOSITION

REAGENT A:

Tris buffer pH 7.5	64 mmol/l
Pyruvate	0.81 mmol/l
Sodium azide	0.095 g/l

REAGENT B:

Good buffer pH 9.6	15 mmol/l
NADH	1.05 mmol/l
Sodium azide	0.095 g/l

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 10 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 2 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 30 days at 2-8 °C, protected from light.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, (heparin or EDTA) plasma.
Do not use haemolysed samples because haemolysis can cause falsely positive results as red blood cells contain high LDH levels.

Loss of activity within 3 days: at 2-8 °C < 8%
at 15-25 °C < 2%
Stability at - 20 °C: at least 3 months.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known LDH activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	340 nm (334 nm, 365 nm)
Optical path	1 cm
Reaction	kinetic (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes :

	Sample
Reagent A	1000 µl
Sample	20 µl
Mix and incubate at 37 °C for 5 minutes, then add:	
Reagent B	100 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Monoreagent procedure

Pipette into disposable or well clean cuvettes :

	Sample
Working reagent	1000 µl
Incubate at 37 °C for 5 minutes, then add:	
Sample	20 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Note

- Reaction volumes can be proportionally changed.
- For values upper than 1000 U/l dilute samples 1+9 with saline solution and multiply result by 10.

CALCULATION OF RESULTS

Activity (U/l) = ΔA/min x factor (f) indicated in the following table:

Bireaegent procedure

340 nm	f = 8888
334 nm	f = 9061
365 nm	f = 16000

Monoreagent procedure

340 nm	f = 8095
334 nm	f = 8252
365 nm	f = 15000

Note:
As the factor "f" used to calculate results depends on several variables (wavelength, temperature, sample volume, reaction volume…), it is recommended to use commercial calibration sera to asset the instruments.

REFERENCE VALUES

Adults: < 480 U/l

Children < 12 years old have LDH levels 10-15% higher than adults ones.

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

		Within Run		Between Run	
Sample	**Mean (U/l)**	**SD**	**%CV**	**SD**	**%CV**
Serum 1	316.6	5.82	1.8	14.81	4.7
Serum 2	508.3	3.94	0.8	12.80	2.5

Linearity
The assay is linear up to 1000 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 4 U/l.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

y = 1.5964x + 0.1344 U/l r = 0.9864

Interferences

Bilirubin	> 40 mg/dl
Triglycerides	> 2000 mg/dl
Ascorbic acid	> 30 mg/dl
Hemoglobin	The presence of hemoglobin in serum indicates destruction of erythrocytes with release of LDH, producing high interference.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

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6. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
7. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

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