

LDH-L Reagent

2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 1200 U/L (20.0 µkat/L)
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.

INTENDED USE

This reagent is intended for the in vitro quantitative determination of LDH (L-Lactate: NAD oxidoreductase EC 1.1.1.27) in human serum on both manual or automated systems.

CLINICAL SIGNIFICANCE

The enzyme Lactate dehydrogenase (LDH) is concentrated in heart, kidney, liver, muscle and body tissues. Consequently, damage to these tissues results in increased serum levels of LDH. Elevated levels are associated with myocardial infarction, renal damage, hepatitis, anaemias, malignancies and muscular disease or damage.¹ There are at least five forms of LDH separable by electrophoresis. The predominant form present varies with the tissue of origin, and therefore has diagnostic value.²

METHODOLOGY

Although the activity of LDH can be measured utilising pyruvate or lactate as a substrate, this reagent uses lactate and is based on the recommendation of the IFCC.³



LDH catalyses the oxidation of the lactate to pyruvate reducing the nicotinamide adenine dinucleotide (NAD) to NADH. The activity of LDH can be determined by the rate of increase in absorbance at 340 nm as NADH is produced.

REAGENT COMPOSITION

Active Ingredient	Concentration
Reagent 1:	
Methyl-D-Glucamine	405 mmol/L
Lithium Lactate	63 mmol/L
Reagent 2:	
NAD	50 mmol/L

pH 9.65 ± 0.1 at 20°C.

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information, consult the LDH-L 2 Part Liquid Reagent Material Data Safety Sheet.

REAGENT PREPARATION

Reagents are supplied ready to use.

STABILITY AND STORAGE

Prior to use:

When stored between 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8°C, the reagents are stable until the expiration date stated on the bottle and kit box label.

Indications of Reagent Deterioration:

- Turbidity; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum²: Use non-haemolysed serum.

Plasma: Not recommended.

Storage²: LDH samples may be stored for at least 1 to 3 days at room temperature (18-25°C) and/or at least 7 days at 4°C. Do not freeze the sample as this will destroy the liver isoenzyme.

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION: CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		
	Reagent 1 (R1)		Reagent 2 (R2)

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Assayed Normal and Abnormal control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available on request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Primary Wavelength	340nm (334- 365nm)
Secondary Wavelength	380 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1:72(R1):18(R2)
eg : Sample Vol	4 µL
Reagent 1 Vol	280 µL
Reagent 2 Vol	70 µL
Delay Time (sample + R1)	≤ 5 minutes
Lag Time (sample + R1 + R2)	> 60 seconds
Read Time	3 - 4 minutes
Reagent Blank Limits	Low 0.0 AU
(340nm, 1cm lightpath)	High 1.0 AU
Linearity	Up to 1200 U/L (20.0µkat/L)
(refer to linearity section)	
Analytical Sensitivity	0.071 ΔmA/min per U/L
(340nm, 1cm lightpath)	(4.25 ΔmA/min per µkat/L)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{\text{SV} \times 6.3 \times \text{P}}$$

Where:

TV	=	Total reaction volume in mL
SV	=	Sample volume in mL
6.3	=	millimolar absorption coefficient of NADH at 340 nm (See note 4).
P	=	Cuvette pathlength in cm.

Example:

ΔAbs/min	=	0.014
Factor	=	14048
LDH	=	0.014 x 14048 = 200 U/L

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.085/min repeat the assay with less sample or dilute with saline. Remember to adjust the factor for the smaller sample volume or multiply the final result by the dilution factor.
3. Valid results depend on accurately calibrated instrument, timing and temperature control.
4. The millimolar absorption coefficient for NADH at 334nm = 6.18 and at 365 nm = 3.40.
5. Unit conversion: U/L x 16.67 x 10⁻³ = µkat/L.

CALIBRATION

Calibration is not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:

Haemoglobin: Avoid the use of haemolysed samples.

Free bilirubin: No interference from free bilirubin up to a level of 278 µmol/L (16.2 mg/dL).

Conjugated bilirubin: No interference from conjugated bilirubin up to a level of 290 µmol/L (17 mg/dL).

Lipaemia: No interference from lipaemia, measured as triglycerides, up to 18.8 mmol/L (1650 mg/dL).

2. Young DS⁴ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES³

At 37°C 114 - 240 U/L (1.90 - 4.01 µkat/L)

The quoted values should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves.⁵

PERFORMANCE DATA

The following data was obtained using the LDH-L 2 Part Liquid Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁶

	LEVEL I	LEVEL II
Within Run		
Number of data points	80	80
Mean (U/L)	142	384
Mean (µkat/L)	2.37	6.41
SD (U/L)	2.1	3.21
SD (µkat/L)	0.035	0.054
CV (%)	1.5	0.8

	LEVEL I	LEVEL II
Total		
Number of data points	80	80
Mean (U/L)	142	384
Mean (µkat/L)	2.37	6.41
SD (U/L)	4.64	10.08
SD (µkat/L)	0.077	0.168
CV (%)	3.3	2.6

METHOD COMPARISON

Comparison studies were carried out using the standard IFCC formulation as a reference. Serum samples were assayed and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	60
Range of sample results	82 to 444 U/L (1.37 to 7.42 µkat/L)
Mean of reference method results	134 U/L (2.24 µkat/L)
Mean of LDH-L results	138 U/L (2.30 µkat/L)
Slope	1.04
Intercept	-2.21 U/L (-0.037 µkat/L)
Correlation coefficient	0.997

LINEARITY

When run as recommended, the assay is linear up to 1200 U/L (20.0 µkat/L).


ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.071 ΔmA/min per U/L (4.25 ΔmA/min per µkat/L).

REFERENCES

1. Searcy, R.L., Diagnostic Biochemistry, McGraw-Hill, New York, NY, 1969.
2. Tietz, N. W., (Ed) Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia, PA, 1976.
3. Bais and Philcox., Eur.J .Clin. Chem. Clin. Biochem. , 1994;32:639.
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5. Wachtel M et al. Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
6. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS; 1984, NCCLS Publication EP5-T.

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840368 (R0)

REF

Reorder Information

Catalogue No.	REAG 1	REAG 2
TR20220	1 x 125 mL	1 x 35 mL
7500 - 120A	4 x 500 mL	
7500 - 220A		2 x 250 mL
TY20201	4 x 53 mL	4 x 15 mL