







LDH-L Reagent

PRODUCT SUMMARY

Stability	:	5 days at 2-8°C
Linear Range	:	20 - 1000 U/L
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of distilled or deionised water.

IVD

SYMBOLS IN PRODUCT LABELLING

EC	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xi - Irritant

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, anaemia's, 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 procedure of Gay, McComb and Bowers.³



LDH catalyses the oxidation of lactate to pyruvate reducing 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
Tris Buffer	100 mmol/L
NAD	7 mmol/L
Lithium Lactate	50 mmol/L
KCl	120 mmol/L
pH 9.0 ± 0.1 at 20°C.	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the LDH-L Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

R36/38 Irritating to eyes and skin.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

REAGENT PREPARATION

Reconstitute the reagent with the volume of distilled or deionized water stated on the vial label. Mix gently until dissolved.

STABILITY AND STORAGE

Prior to use:

When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:

When stored capped at 2-8°C, the reagent is stable for at least 5 days.

Indications of Reagent Deterioration:

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

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

Plasma: Not recommended.

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

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyzer specific consumables, eg: sample cups.
- Distilled or deionized water for reagent preparation and related equipment eg: pipettes.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

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

SYSTEM PARAMETERS

Temperature	30°/37°C
Wavelength	340 nm (334 - 365nm)
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 60
eg: Sample Vol	0.025 mL
Reagent Vol	1.5 mL
Delay/Lag Time	30 seconds
Read Time	60 seconds
Reagent Blank Limits (340nm, 1cm lightpath)	Low 0.0 AU High 2.0 AU
Linearity (refer to linearity section)	20 - 1000 U/L
Sensitivity (340nm, 1cm lightpath)	0.103 ΔmA/min per U/L

CALCULATIONS

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

Activity in U/L = ΔAbs/min x Factor

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

Where:

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

Example:

ΔAbs/min	=	0.015
Factor	=	9683
LDH	=	0.015 x 9683 = 145 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 0.10 /min, dilute with saline and reassay. Multiply the final result by the dilution factor.
3. Valid results depend on accurately calibrated instruments, timing and temperature control.
4. The millimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
5. Unit conversion: $\text{U/L} \times 16.67 \times 10^{-3} = \mu\text{kat/L}$.

CALIBRATION

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 every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established range indicates 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 the local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on a well maintained automated clinical chemistry analyzer. The following results were obtained:
Haemoglobin: No interference from haemoglobin up to 500 mg/dL.
Bilirubin: No interference from bilirubin up to 510 $\mu\text{mol/L}$ (30 mg/dL).
Lipaemia: No interference from lipaemia, measured as triglycerides, up to 11.4 mmol/L (1000 mg/dL).
2. Young DS⁴ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁵

At 37°C: 114 to 240 U/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.⁶

PERFORMANCE DATA

The following data was obtained using the LDH-L reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁷

	LEVEL I	LEVEL II
Mean (U/L)	120	526
CV (%) Within run	2.3	1.3
CV (%) Between day	3.2	1.9

ACCURACY

Comparison studies were carried out using another commercially available method 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	85 - 696 U/L
Mean of reference method results	169 U/L
Mean of LDH-L results	172 U/L
Slope	0.99
Intercept	4.4 U/L
Correlation coefficient	0.997

LINEARITY

When run as recommended, the assay is linear between 20 and 1000 U/L (0.33 and 16.67 $\mu\text{kat/L}$).

SENSITIVITY

When run as recommended the sensitivity of this assay is 0.103 $\Delta\text{mA/min}$ per U/L.

REFERENCES

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6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
7. 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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REF

Reorder Information

Catalogue No. Configuration

TR20015 20 x 20 mL