3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

L-Lactic Dehydrogenase
from bovine heart

Catalog Number **L3916**
Storage Temperature 2–8 °C

CAS RN 9001-60-9
EC 1.1.1.27
Synonyms: (*S*)-Lactate:NAD^+ oxidoreductase, L-LDH, LAD, LD

Product Description

Lactic dehydrogenase may be used for the quantitative, enzymatic determination of either lactate or pyruvate. The reversible conversion of lactate to pyruvate is catalyzed by lactic dehydrogenase and is driven to completion by excess of nicotinamide adenine dinucleotide oxidized form (NAD) or nicotinamide adenine dinucleotide reduced form (NADH).

$$\text{Pyruvate} + \text{NADH} \xleftrightarrow{\text{LHD}} \text{Lactate} + \text{NAD}$$
(High A_{340}) (Low A_{340})

Early analytical methods for lactate lacked both specificity and sensitivity. The availability of highly purified nicotinamide adenine dinucleotide (NAD) and lactate dehydrogenase (LDH) led to a highly specific assay for this metabolite.[1] The catalytic action of LDH permits measurement of lactate in terms of the generation of NADH spectrophotometrically at 340 nm. In the presence of excess NADH, effectively all pyruvate is converted to lactate. The reduction of absorbance at 340 nm due to oxidation of NADH to NAD allows the calculation of the original amount of pyruvate present.

This LDH product is provided as a suspension in 2.2 M ammonium sulfate with ~1,000 units/mL.

Unit definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37 °C.

Precautions and Disclaimer

This product is for R&D use only, not for *in vitro* diagnostic use, drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Mix the product by gentle inversion until suspension is uniform before removing an aliquot.

Biological sample preparation

Lactate/pyruvate may be determined in biological samples, blood, plasma, or cerebrospinal fluid. Large and variable changes in lactate and pyruvate concentrations in blood may occur after specimen collection.[2,3] If blood is permitted to stand before preparing the protein-free solution, the lactate concentration will increase as a result of glucose metabolism. Use of fluoride/oxalate as anticoagulant can minimized this increase in lactate concentration. Blood collected with heparin or EDTA must be deproteinized immediately as large increases in lactate concentrations will otherwise occur. There appears to be no adequate means of stabilizing pyruvate levels except by immediate precipitation of blood proteins.

Plasma may be substituted for blood if separated within 1 hour from blood, which has been collected in fluoride/oxalate and kept on ice. Plasma lactate levels have been found to be stable up to 48 hours stored at 2–8 °C or for at least one week stored frozen. If plasma is to be used for lactate determination, simply substitute 2.0 mL of plasma for the 2.0 mL of whole blood in step 2 of the deproteinization procedure.

Notes: The volumes of sample and deprotinization reagent may be proportionally decreased. For example, 0.2 mL of blood or cerebrospinal fluid may be added to 0.4 mL of deproteinizing agent. This will provide sufficient clear supernatant from which 0.1 mL can be withdrawn for the lactate assay.

A second centrifugation of the supernatant may be necessary to obtain a clear protein-free solution.

If the protein-free solution is to be obtained by filtration, rather than centrifugation, use 3.0 mL of blood and 6.0 mL of cold deproteinizing agent to obtain sufficient filtrate.

Lactate concentrations in the TCA or PCA supernatants are stable for at least 1 week stored at 2–8 °C. Freezing may extend stability.

Reagents Required but not Provided for Sample Deproteinization

- Perchloric Acid (PCA), 8% (w/v) - Prepare by diluting 7 mL of 70% (w/w) perchloric acid (Catalog Number 244252, ACS Reagent) to 100 mL with water.
  Caution: Perchloric acid solutions can become explosive if allowed to dry. Always rinse glassware and working surfaces with a large volume of water.

- OR Alternative reagent for lactate analysis only Trichloroacetic Acid (TCA, Catalog Number T0199) – 0.6 N TCA, ~10% (w/v)

Sample Deproteinization Procedure

1. Draw blood with minimum of stasis and transfer it immediately to a chilled tube or flask.

2. Pipette 2.0 mL of blood into a centrifuge tube containing 4.0 mL of cold 8% perchloric acid (for pyruvate or lactate analysis samples) or 4.0 mL of 10% trichloroacetic acid (for lactate analysis samples only). Vortex the mixture for ~30 seconds. Keep the blood-precipitate mixture cold for an additional 5 minutes to ensure complete protein precipitation. Centrifuge 10 minutes at ~1,500 × *g*.

3. The clear TCA or PCA supernatant is ready for use.

Storage/Stability

Store the product at 2–8 °C.

Procedures

A. Lactate Determination

To measure lactate, the reaction contains excess NAD and glycine-hydrazine buffer. To force the reaction to completion in this direction, it is necessary to trap the formed pyruvate with hydrazine. The increased absorbance at 340 nm due to NADH formation is proportional to the lactate originally present.

Reagents and Equipment Required but not Provided for Lactate Determination

- Glycine buffer (Catalog Number G5418) - 0.6 M Glycine and 0.5 M hydrazine, pH 9.2.
- Nicotinamide adenine dinucleotide (NAD, Catalog Number N8285) - 10 mg preweighed vials
- Spectrophotometer capable of accurately measuring absorbance at 340 nm
- Cuvettes with optical properties suitable for use at 340 nm
- Pipetting devices for the accurate delivery of volumes required for the assay
- Water bath, 37 °C
- Centrifuge

1. Prepare Reaction Mixture - Reconstitute the appropriate number of NAD vials (Catalog Number N8285) required by pipetting the following reagents into each vial:

   2.0 mL Glycine buffer (Catalog Number G5418)
   4.0 mL water
   0.1 mL L-Lactate dehydrogenase (Catalog Number L3916)

   Cap and invert the vials several times to dissolve the NAD. Combine contents of vials if more than one are being used. The Reaction Mixture remains active for 4 hours at room temperature or 24 hours at 2–8 °C.
   Note: Do **NOT** use 1.5 M Trizma Base Solution in place of Glycine buffer (Catalog Number G5418).

2. Pipette 2.9 mL of the Reaction Mixture into cuvettes labeled BLANK and TEST.

3. To the cuvette labeled BLANK, add appropriate blank solution (water, 0.1 mL 10% TCA, or 8% PCA if used; see Biological Sample Preparation). Mix gently by inversion.

4. To cuvette labeled TEST add 0.1 mL of biological sample. Mix gently by inversion.

5. Incubate cuvettes labeled BLANK and TEST for 15 minutes at 37 °C or for 30 minutes at 25 °C.

6. Read and record absorbance of TEST solution at 340 nm vs. the BLANK solution as reference. Complete readings within 10 minutes.

Calculation

$$\text{Lactate (mM)} = \Delta A_{340} \times 14.5 = \frac{\Delta A_{340} \times 3.0}{6.22 \times 0.0333 \times 1}$$

ΔA_{340} = Final maximum absorbance at 340 nm
3.0 = Reaction volume (mL)
6.22 = Millimolar extinction coefficient of NADH at 340 nm
0.0333 = Volume (mL) of sample in cuvette
1 = Lightpath (cm).
If a lightpath other than 1 cm is used, substitute value (cm) into equation.

Above calculations are valid only if a narrow-bandwidth spectrophotometer is employed. If a wide-bandwidth instrument, which does not provide a linear response at 340 nm is used, a calibration curve is recommended.

The prepared Reaction Mixture will conveniently handle lactate concentrations of ≤13.3 mM. If a higher level is observed or expected, use proportionately less sample in step 4 of Lactate Determination. Make up the difference in volume with water and adjust calculations accordingly. The lowest practical level of lactate that can be measured by this method is considered to be 0.22 mM.

B. Pyruvate Determination

To measure pyruvate, the reaction contains excess NADH. The reduction in absorbance at 340 nm due to oxidation of NADH to NAD becomes a measure of pyruvate originally present.

Reagents and Equipment Required but not Provided for Pyruvate Determination

- 1.5 M Trizma® base solution (Tris(hydroxymethyl)-aminomethane, Catalog Number T1699) - Contains 0.05% sodium azide as preservative.
- Nicotinamide adenine dinucleotide, reduced form, disodium salt (NADH, Catalog Number N0786) - 1 mg preweighed vials
- Spectrophotometer capable of accurately measuring absorbance at 340 nm
- Cuvettes with optical properties suitable for use at 340 nm
- Pipetting devices for the accurate delivery of volumes required for the assay
- Centrifuge

1. Prepare NADH Solution - Pipette 2.2 mL of 1.5 M Trizma Base Solution (Catalog Number T1699) into an NADH vial (Catalog Number N0786). This is sufficient for 4 tests.
   Note: Do **NOT** use Glycine buffer (Catalog Number G5418) in place of 1.5 M Trizma Base Solution.

2. Pipette the following into a 1 cm lightpath cuvette:

   2.0 mL of biological sample
   0.5 mL of 1.5 M Trizma Base Solution (Catalog Number T1699)
   Note: Do **NOT** use Glycine buffer (Catalog Number G5418) in place of 1.5 M Trizma Base Solution.

   Mix by swirling or inversion.
   Note: Mixing is essential. Mixture must be brought to proper pH by thorough mixing before adding NADH Solution.

3. Add 0.5 mL of NADH Solution prepared in step 1. Invert cuvette several times to mix.

4. Read and record absorbance at 340 nm vs. water as reference. This is INITIAL A_{340}.
   Note: The INITIAL A_{340} value should be greater than 0.6 using a 1 cm cuvette.

5. To the same cuvette quickly add 0.05 mL of L-Lactic dehydrogenase (Catalog Number L3916). Invert several times to mix.
   Note: Elapsed time between steps 4 and 5 should be kept to a minimum.

6. After 2–5 minutes, again read and record the absorbance at 340 nm. This is FINAL A_{340} reading. After an additional 5 minutes, reread the solution at 340 nm to assure a constant minimum value has been reached. Reaction may be considered complete when the absorbance decrease is less than 0.001/minute.

7. Determine absorbance change:
   ΔA_{340} = INITIAL A_{340} – FINAL A_{340}

Calculations

$$\text{Pyruvate (mM)} = \Delta A_{340} \times 0.723 = \frac{\Delta A_{340} \times 3.0}{6.22 \times 0.667 \times 1}$$

ΔA_{340} = INITIAL A_{340} – FINAL A_{340}
3.0 = Reaction volume (mL)
6.22 = Millimolar extinction coefficient of NADH at 340 nm
0.667 = Volume (mL) of sample in cuvette
1 = Lightpath (cm).
If a lightpath other than 1 cm is used, substitute value (cm) into equation.

Above calculations are valid only if the spectrophotometer produces a linear absorbance response at 340 nm. If a nonlinear response is obtained, use of a calibration curve is recommended.

The reaction mixture prepared above will conveniently handle pyruvate concentrations of ≤0.34 mM. If the concentration of pyruvate exceeds 0.34 mM, repeat the test using a 2-fold dilution of the sample with water. Multiply the result by 2 in the final calculation to adjust for the concentration change. The lowest practical level of pyruvate that can be measured using this method is estimated to be 0.01 mM.

The dilution effect caused by the addition of the lactic dehydrogenase in the reaction mixture is disregarded.

Results

Several investigators[1,4–10] have studied the specificity of the lactate dehydrogenase reaction in terms of possible interference by various α and β-keto and hydroxy acids. Included among those acids studied were malate, glyoxylate, α-ketobutyrate, oxalacetate, acetoacetate, α and β-hydroxybutyrate, glycerate, β-hydroxypyruvate, and phenylpyruvate. Some of these compounds were found to act as substrates to varying degrees for LDH. However, in practically all cases, it was reported they were rarely present in significant concentrations in biologic fluids or else yielded substrate turnover rates too slow to cause any significant interference.

References

1. Gloster, J.A., and Harris, P., Observations on an enzymatic method for the estimation of pyruvate in blood. Clin. Chim. Acta, **7**, 206 (1962).
2. Long, C., The stabilization and estimation of pyruvic acid in blood samples. Biochem. J., **38**, 447 (1944).
3. Pearce, P.J., Concerning the stability of pyruvate and citrate in fluorided shed blood. Vet. Rec., **73**, 341 (1961).
4. Marbach, E.P., and Weil, M.H., Rapid enzymatic measurement of blood lactate and pyruvate. Clin. Chem., **13**, 314 (1967).
5. Loomis, M.E., An enzymatic fluorometric method for the determination of lactic acid in serum. J. Lab. Clin. Med., **57**, 966 (1961).
6. Segal, S. et al., An enzymatic spectrophotometric method for the determination of pyruvic acid in blood. J. Lab. Clin. Med., **48**, 137 (1956).
7. Meister, A., Reduction of α,γ-diketo, and α-keto acids catalyzed by muscle preparations and by crystalline lactic dehydrogenase. J. Biol. Chem., **184**, 117 (1950).
8. Bucher, T. et al., Pyruvate. in Methods of Enzymatic Analyses, Bergmeyer, H.U., ed., Academic Press (New York, NY:1963) pp 253–259.
9. Schon, R., A simple and sensitive enzymatic method for the determination of L(+)lactic acid. Anal. Biochem., **12**, 413 (1965).
10. Fleischer, W.R., Enzymatic Methods for Lactic and Pyruvic Acids. in Standard Methods of Clinical Chemistry, Vol. 6, MacDonald, R.P., ed., Academic Press (New York, NY:1970) pp 245–259.

VNC,MAM 10/10-1