Originating Department	QC
Approval Departments	QA, QC & Validation
Effective Date	Refer to Q-pulse

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Lactate Dehydrogenase

1.2 **Systematic Name**: (S) – Lactate: NAD^+ oxidoreductase

1.3 **E.C. Number**: 1.1.1.27

1.4 **Source**: Porcine heart, rabbit muscle, bovine heart, porcine muscle

2.0 ASSAY PRINCIPLE[1]

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

The rate of decrease of absorbance at 340nm is a measure of the LDH activity.

3.0 UNIT DEFINITION

That amount of enzyme causing the oxidation of one micromole of NADH per minute at 25°C and pH 7.4

4.0 EQUIPMENT REQUIRED

Double beam UV/vis spectrophotometer with chart recorder
Water bath set to achieve a reaction temperature of 25°C (± 0.1°C)
Thermometer
Silica and plastic cuvettes
Test tubes
Pipettes and tips

5.0 REAGENTS REQUIRED

When using the following reagents, refer to the manufacturer's instructions for safe handling and disposal.

Reagent details

Chemical / Reagent	Supplier	Product No.	F.W.
Di-potassium hydrogen phosphate	VWR	26931.263	174.18
Potassium dihydrogen phosphate	VWR	26936.293	136.09
Bovine serum albumin	Sigma-Aldrich	10735108001	N/A
NADH disodium salt	Sigma-Aldrich	10128023001	709.4
Sodium Pyruvate	Sigma-Aldrich	P8574	110.04

6.0 PREPARATION OF REAGENTS

6.1 0.05M Potassium phosphate pH 7.4

Dissolve 8.71g of di-potassium hydrogen phosphate in water and adjust to a final volume of 1000ml.

Dissolve 3.40g of potassium di-hydrogen phosphate in water and adjust to a final volume of 500ml.

Titrate the di-potassium hydrogen phosphate with the potassium di-hydrogen phosphate to obtain a pH of 7.4.

From 1.0M stock buffers:

Add 39mL of 1M di-potassium hydrogen phosphate and 11mL of 1M potassium di-hydrogen phosphate and make up to a final volume of 1L.

Stable for 2 weeks at 2 to 8°C

6.2 Diluent buffer (0.05M Potassium phosphate pH 7.4 containing 0.1% BSA)

Add 500mg of Bovine serum albumin to 500ml of 0.05M potassium phosphate pH7.4. Allow 10 minutes for the BSA to soak into the buffer and then gently stir to avoid foaming.

Stable for 5 days at 2 to 8°C

6.3 Buffered water (0.005M Potassium phosphate pH 7.4)

Add 10ml of 0.05M potassium phosphate pH 7.4 to 90ml of water.

Prepare fresh daily.

6.4 0.006M NADH solution

Weigh approximately 15mg of NADH into a new glass vial and dissolve up to a concentration of 4.25mg/ml in buffered water.

Stable for 3 days at 2 to 8°C

6.5 0.023M Sodium pyruvate solution

Weigh approximately 15mg of Sodium pyruvate into a new glass vial and dissolve to a concentration of 2.5mg/ml in buffered water.

Prepare fresh daily.

6.6 Enzyme solution

Freeze-dried powders:

Into new glass vials accurately weigh at least 10mg of freeze-dried powder, each test sample to be weighed in triplicate. Dissolve each to a concentration of 5mg/ml in 0.05M potassium phosphate pH 7.4. Immediately prior to assay, dilute to approximately 0.25 U/ml in enzyme diluent.

Liquid preparations:

Immediately prior to assay, dilute to approximately 0.25 U/ml in enzyme diluent.

Process Samples

Immediately prior to assay, dilute to approximately 0.25 U/ml in enzyme diluent, ensuring the concentration is within the range of 0.0500 U/ml to 0.375 U/ml (equivalent to reaction rates (ΔA_{340}/min) of 0.0114 to 0.0778)[1].

7.0 TEST PROCEDURE

Temperature = 25°C Wavelength = 340nm Light path = 10mm

Into disposable test tubes pipette the following:

	Test	**Reference**
0.05M Potassium phosphate pH 7.4	2.70ml	2.80ml
0.006M NADH	0.10ml	0.10ml
0.023M Sodium Pyruvate	0.10ml	0.10ml

Allow the solutions to equilibrate to 25°C for approximately 5 minutes, then add:

Enzyme solution, diluted to ~0.25U/ml:	0.10ml	0.00ml
Total volume (V_t):	3.00ml	3.00ml

Transfer to plastic cuvettes and record the decrease in absorbance at 340nm, reading the test solution versus the reference solution for approximately 5 minutes. Measure the change of absorbance per minute (ΔA_{340}/min) over the linear portion of the curve and use this value in the calculation.

[1] Taken from Analytical Test Method Validation (ATMV 030)

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\frac{\Delta A_{340}/\text{min} \times V_t \times \text{dilution factor}}{V_s \times \varepsilon}$

Where: V_t = final volume of the reaction mix = 3.00ml
V_s = sample volume = 0.10ml
ε = micromolar extinction coefficient for NADH (cm^2/µmole) = 6.22

Volume activity (U/ml) = ΔA_{340}/min x 4.82 x dilution factor

8.2. For freeze-dried samples: Weight activity (U/mg material) = $\frac{\text{U/ml}}{\text{mg/ml}}$

Specific activity (U/mg protein) = $\frac{\text{U/mg material}}{\text{mg protein/mg material}}$

8.3 For liquid preparations:

Specific activity (U/mg protein) = $\frac{\text{U/ml}}{\text{mg protein/ml}}$

9.0 PROTEIN DETERMINATION

Protein is determined by the method of Lowry et al in accordance with Analytical Procedure AP62[2].

10.0 $A_{280}^{1\%}$ DETERMINATION

This is determined in accordance with Analytical Procedure AP63

11.0 ASSOCIATED DOCUMENTS

AP62	Lowry Protein Determination
AP63	Spectrophotometric Measurements
ATMV-030	Validation of Lactate Dehydrogenase Assay

12.0 REFERENCES

1. Reeves, W.J., & Fimognari, G.M. (1963) *J. Biol. Chem.* **238**, 3583

2. Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951) *J. Biol. Chem*. **193**, 265

13.0 REVISION HISTORY

Document version number	Section number	Summary of Changes
04	Global	Header & footer changed; approval/effective date changed to refer to Q-pulse; Changes for document version 03 removed. Layout adjusted for ease of use.
	4.0	Added plastic to cuvettes
	5.0	Updated suppliers and product numbers to reflect current suppliers
	6.1	Grammatical error: changed buffer volume and amended weight. Preparation of buffer from stock buffer added
	6.6	Added in process sample dilution data from validation
	7.0	Corrected molarity on the potassium phosphate used in the reaction.
	11.0	New section for associated documents added