Originating Department	QC
Approval Departments	QC & Validation
Effective Date	Refer to Q-Pulse

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Malate Dehydrogenase

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

1.3 **E.C. Number**: 1.1.1.37

1.4 **Source**: Porcine heart

2.0 ASSAY PRINCIPLE[1]

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} (S)\text{-Malate} + NAD^+$$

The rate of decrease of absorbance at 340nm is a measure of the MDH 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 cuvettes
Test tubes
Manual pipettes and tips

5.0 REAGENTS REQUIRED

When using hazardous chemicals, handle in accordance with COSHH Regulations.

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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 (BSA)	Sigma	10735108001	N/A
NADH disodium salt	Sigma	10128023001	709.4
Oxaloacetic acid	Sigma	O4126	132.07

6.0 PREPARATION OF REAGENTS

6.1 0.1M potassium phosphate pH7.4

Dissolve 17.42g of di-potassium hydrogen phosphate in water and adjust to a final volume of 1000ml.

Dissolve 6.80g 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.
Stable for 2 weeks at 2 to 8°C

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

Add 500mg of Bovine serum albumin to 500ml to 0.1M potassium phosphate pH7.4. Allow to soak for approximately 10 minutes. Stir gently to avoid foaming.
Stable for 2 weeks at 2 to 8°C

6.3 Buffered water (0.005M Potassium phosphate pH 7.4)

Add 5ml of 0.1M potassium phosphate pH 7.4 to 95ml of water.
Prepare fresh daily.

6.4 0.006M NADH solution

Weigh approximately 15mg of NADH into a new glass vial and dissolve to a concentration of 4.25mg/ml in buffered water. Store in a dark bottle.
Stable for 3 days at 2 to 8°C

6.5 0.006M Oxaloacetic acid solution

Weigh approximately 15mg of Oxaloacetic acid into a new glass vial and dissolve to a concentration of 0.79mg/ml in buffered water. Store in a dark bottle.
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 0.1M potassium phosphate pH7.4. Immediately prior to assay, dilute to approximately 0.25 U/ml in 0.1M potassium phosphate pH7.4.

Liquid preparations:

Immediately prior to assay, dilute to approximately 0.25 U/ml in 0.1M potassium phosphate pH7.4.

7.0 TEST PROCEDURE

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

Into disposable test tubes pipette the following:

	Test	Reference
0.1M Potassium phosphate pH 7.4	2.70ml	2.80ml
0.006M NADH	0.10ml	0.10ml
0.006M Oxaloacetic acid solution	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 silica 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.

8.0 CALCULATION

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

Where: V_t = final volume of the reaction mix = 3.00ml
V_s = sample volume = 0.10ml
ε = micromolar extinction coefficient for NADH ($cm^2/\mu mole$) = 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) = $\dfrac{\text{U/ml}}{\text{mg/ml}}$

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

8.3 For liquid preparations:

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

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
MST060	Malate Dehydrogenase Codes MDHP3 and MDHP3G

12.0 REFERENCES

1. Mehler, A.H., Kornberg, A., Grisolia, S., & Ochoa, S. (1948) *J. Biol. Chem.* **179**, 961

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	Changed Header to reflect current practice; approval date removed and effective date changed to 'Refer to Q-Pulse'. Changes for document version 03 removed. Corrected grammatical errors.
	5.0	Reagent Details- Updated supplier and product numbers for NADH and BSA; Corrected FW for oxaloacetic acid.
	6.1	Changed volume of 0.1M di-potassium hydrogen phosphate to be prepared from 500ml to 1L. Weight corrected from 8.71g to 17.42g.
	6.4	Added store in a dark bottle.
	11.0	MST060 added to associated documents.