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

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Glutamate dehydrogenase

1.2 **Systematic Name**: L-Glutamate: NAD(P)$^+$ oxidoreductase (deaminating)

1.3 **E.C. Number**: 1.4.1.3

1.4 **Source**: Bovine liver

2.0 ASSAY PRINCIPLE

The oxidation of NADH is measured in the presence of 2-Oxoglutarate and ammonium ions with no added ADP.

$$\text{2-Oxoglutarate} + NH_3 + \text{NADH} + H^+ \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

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

3.0 UNIT DEFINITION

That amount of enzyme which causes the transformation of one micromole of 2-Oxoglutarate per minute at 25°C and pH 7.3 (under assay conditions not containing ADP).

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 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.
Imidazole	Fisher Scientific	I/0010/53	68.08
Hydrochloric acid (6M)	Fisher Scientific	72033-1L	N/A
Bovine serum albumin (BSA)	Sigma-Aldrich	10735108001	N/A
α-Ketoglutaric acid	Sigma-Aldrich	K1750	146.1
Sodium hydroxide (2M)	Fisher Scientific	71474-1L	N/A
Ammonium acetate	VWR	21200.264	77.08
Potassium dihydrogen phosphate	VWR	26936.293	136.09
Di-Potassium hydrogen phosphate	VWR	26931.263	174.18
Nicotinamide adenine dinucleotide (reduced) disodium salt (NADH)	Sigma-Aldrich	10128023001	709.41
Ethylenediamine tetra-acetic acid disodium salt dihydrate (EDTA)	Sigma-Aldrich	ED2SS	372.24

6.0 PREPARATION OF REAGENTS

6.1 6M Hydrochloric acid

Use as required and refer to the manufacturer's expiry date.

6.2 2M Sodium hydroxide

Use as required and refer to the manufacturer's expiry date.

6.3 0.058M Imidazole/HCl pH 7.3

Combine 58mL of 1M Imidazole with 3.3mL of 6M HCl and make to a final volume of 1L with water.

Stable at 2 to 8°C for 5 days.

6.4 Diluent buffer: 0.058M Imidazole/HCl pH 7.3 containing 0.10% BSA.

Add 500mg of BSA to 500ml of 0.058M Imidazole/HCl, pH 7.3. Allow 10 minutes for the BSA to soak into the buffer and then gently stir to avoid foaming.

Stable at 2 to 8°C for 5 days.

6.5 0.05M Potassium phosphate pH 7.4

Combine 39mL of 1M di-potassium hydrogen phosphate with 11mL of potassium di-hydrogen phosphate and make to a final volume of 1L with water.

Stable for 2 weeks at 2 to 8°C.

6.6 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.

6.7 0.13M α-Ketoglutarate, (2-Oxoglutarate), pH 6.0 - 7.0

Prepare α-Ketoglutarate to a concentration of 19mg/mL with 2M NaOH added at 0.127mL per mL of final volume.

e.g. Assuming 500mg of α-Ketoglutarate weighed then final volume would be 26.3mL (500 ÷ 19).

2M NaOH to be added at 0.127mL per mL of final volume which equates to 3.34mL (26.3 x 0.127).

Therefore, dissolve the 500mg of α-Ketoglutarate in 23mL (26.3 – 3.34) of water and add 3.34mL of 2M NaOH.

Stable for 5 days at 2 to 8°C.

6.8 0.004M NADH

Weigh approximately 14mg of NADH into a new glass vial. Dissolve up to a concentration of 2.84mg/ml in buffered water. Store in a dark bottle.

Stable for 5 days at 2 to 8°C.

6.9 1.6M Ammonium acetate / 4mM EDTA

Dissolve 2.46g of Ammonium acetate and 29.8mg of EDTA in 15ml of water. Once dissolved make up to a final volume of 20mL.

Stable for 1 week at 2 to 8°C.

6.10 Enzyme solution

Freeze-dried powders:
Accurately weigh at least 10mg into new glass vials, each 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 0.05M potassium phosphate pH 7.4

Liquid Preparations:
Immediately before use dilute to approximately 0.25U/ml in enzyme diluent buffer.
Process samples:
Dilute to approximately 0.25 U/ml, ensuring the concentration is within the range of 0.0505U/ml to 0.373 U/ml (equivalent to reaction rates (ΔA_{340}/min) of 0.010 to 0.077)[1]

7.0 TEST PROCEDURE

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

Into disposable test tubes pipette the following:

	TEST	REF
0.058M Imidazole/HCl pH 7.3	2.40ml	2.40ml
0.13M α-Ketoglutarate pH 6.0-7.0	0.30ml	0.30ml
0.004M NADH	0.10ml	0.10ml
Ammonium acetate/EDTA solution	0.10ml	0.10ml
Diluent buffer	0.00ml	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
	3.00ml	3.00ml

Transfer to disposable 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 (ml) = 3.00
V_s = sample volume (ml) = 0.10
ε = micromolar extinction coefficient for NADH (cm^2/µmole) = 6.22

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

[1] Taken from Analytical Test Method Validation (ATMV 011)

8.2. For freeze-dried samples:

$$\text{Weight activity (U/mg material)} = \frac{\text{U/ml}}{\text{mg/ml}}$$

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

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[1].

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

This is determined in accordance with Analytical Procedure AP63

11.0 ASSOCIATED DOCUMENTS

ATMV011	Analytical Test Method Validation for Glutamate Dehydrogenase
AP62	Lowry Protein determination
AP63	Spectrophotometric measurements
MST073	Macro Spreadsheet Template for Glutamate Dehydrogenase

12.0 REFERENCES

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

13.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
10	Header/Footer	Version changed to 10, BBI group removed from footer
	Global	Review history for versions 7, 8 & 9 removed for clarity
	6.9	Updated as per change request CR18600, to describe best practice.