Originating Department	QC
Approval Departments	QA, QC, T&V
Approval Date	19th July 2017
Effective Date	21st July 2017

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Urease

1.2 **Systematic Name**: Urea amidohydrolase

1.3 **E.C. Number**: 3.5.1.5

1.4 **Source**: Jack Bean

2.0 ASSAY PRINCIPLE

The method of assay is based on that described by Schiegel and Kaltwasser[1] in which the ammonia released during the enzyme catalysed hydrolysis of urea is measured via the glutamate dehydrogenase coupled reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

$$NH_3 + \alpha\text{-Ketoglutarate} + \text{NADH} + H^+ \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+$$

The rate of decrease in absorbance at 340nm per unit time is a measure of the Urease activity.

3.0 UNIT DEFINITION

That amount of enzyme causing the conversion of one micromole of Urea per minute at 25˚C and pH 8.0

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

Reagent details

Chemical / Reagent	Supplier	Product No.	F.W.
6M Hydrochloric acid	Sigma	72033	N/A
2M Sodium hydroxide	Sigma	71474	N/A
Potassium dihydrogen phosphate	VWR	26936.293	136.09
di-Potassium hydrogen phosphate	VWR	26931.293	174.18
Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA)	Sigma	ED2SS	372.2
Tris	VWR	17-1321-01	121.14
NADH, disodium salt	Roche	10 128 023 001	709.4
α-Ketoglutaric acid	Sigma	K1750	146.1
Urea	VWR	28877.235	60.06
Glutamate dehydrogenase (GLDH)	BBI Solutions	GDHB3	N/A

6.0 PREPARATION OF REAGENTS

6.1 6M Hydrochloric acid

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

6.2 2M Sodium hydroxide

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

6.3 0.05M Potassium Phosphate pH 7.4

Dissolve 0.871g di-Potassium hydrogen phosphate in water and adjust to a final volume of 100ml.

Dissolve 0.340g of Potassium di-hydrogen phosphate in water and adjust to a final volume of 50ml.

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

6.4 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

6.5 0.05M Tris/HCl/0.001M EDTA pH 8.0

Dissolve 6.06g of Tris in approximately 800ml of analytical grade water. Add 0.370g of EDTA and stir until dissolved. Adjust to pH 8.0 with 6M Hydrochloric acid and adjust to a final volume of 1L with water.
Stable for 2 weeks at 2 to 8˚C

6.6 0.0085M NADH solution

Weigh approximately 30mgs of NADH into a new glass vial and dissolve to a concentration of 6.0mg/ml in buffered water. Store in a dark bottle.
Stable for 5 days at 2 to 8˚C

6.7 0.025M α-Ketoglutarate

Dissolve 91.3mg of α-Ketoglutaric acid in approximately 10ml of water. Adjust to pH 5.0 with 2M Sodium hydroxide. Adjust to a final volume of 25ml with water. Store in a dark bottle.
Stable for 5 days at 2 to 8˚C

6.8 0.3M Urea

Weigh approximately 90mg of Urea into a new glass vial and dissolve to a concentration of 18mg/ml in water.
Prepare fresh daily

6.9 GLDH Solution (250U/ml)

Weigh approximately 50mg GLDH into a new glass vial and dissolve to a concentration of 250U/ml in 0.05M potassium phosphate pH 7.4.
Prepare fresh daily

6.10 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 Tris/HCl/0.001M EDTA pH 8.0. Immediately prior to assay, dilute to approximately 0.10U/ml in 0.05M Tris/HCl/0.001M EDTA pH 8.0.

Liquid preparations:

Immediately prior to assay, dilute to approximately 0.1 U/ml in 0.05M Tris/HCl/0.001M EDTA pH 8.0.

7.0 TEST PROCEDURE

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

Into disposable test tubes pipette the following:

0.05M Tris/HCl/0.001M EDTA pH 8.0	5.00ml
0.025M α-Ketoglutarate	0.20ml
0.0085M NADH	0.20ml

Allow solutions to equilibrate to 25°C, mix well then transfer 2.70ml to the test cuvette and the remainder to the blank cuvette.

To the test cuvette add:

GLDH solution (250U/ml)	0.10ml
Enzyme solution, diluted to ~0.10U/ml	0.10ml

Mix with a cuvette stirrer then record the change in absorbance at 340nm, reading the test solution versus the reference solution for 2-3 minutes. If a blank rate remains, discard solutions and repeat. If a blank rate is still present prepare fresh reagents.

To the test cuvette add:

0.3M urea	0.10ml
Total volume (V_t) =	3.00ml

Mix with a cuvette stirrer then record the change in absorbance at 340nm, reading the test solution versus the reference solution for approximately 10 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) = $\dfrac{\Delta A_{340}/\text{min} \times V_t \times \text{dilution factor}}{2 \times V_s \times \varepsilon}$

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
2 = factor to allow for 2μmoles of Ammonia produced from 1μmole of Urea

Volume activity (U/ml) = ΔA_{340}/min x 2.41 x dilution factor

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

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

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

12.0 REFERENCES

1. Schlegel, H. and Kaltwasser, H. (1974) *Methods of Enzymatic Analysis, 2nd Edition.* **2**, 1081

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
03	Global	Reformat throughout
	1.5	Section deleted to avoid unnecessary document updates when enzyme codes are changed or removed
	5.0	Reagent details amended to reflect current suppliers
	6.3	Reference to analytical grade water removed Calculation amendment of 1.14g to 0.87g Order of reagents corrected to reflect the correct way to titrate to make buffer
	6.4	Reference to analytical grade water removed Reference to cooling buffered water until a thin film of ice forms removed to reflect current practice
	6.5	Reference to analytical grade water removed 1000ml changed to 1L
	6.6	Into a new glass vial added, store in a dark bottle added
	6.7	Reference to analytical grade water removed, store in a dark bottle added
	6.8	Into a new glass vial added Dissolve to a concentration added Reference to analytical grade water removed Temperature removed to reflect current practice
	6.9	Into a new glass vial added Temperature removed to reflect current practice
	6.10	Section split into Freeze-dried powders and liquid preparations Unused vials changed to new glass vials Approximately 20mg changed to at least 10mg 1mM changed to 0.001M Reference to storing on ice and using within an hour removed to reflect current practice
	7.0	Light path changed to 10mm Test tubes replaced with disposable test tubes Temperature of test tubes removed The corrected to then
	8.0	Section 8.0 separated into 2 parts
	11.0	New section for Associated Documents added
	11.0 (now 12.0)	References reformatted