Enzymatic assay for the determination of urea and ammonia in foodstuff and other sample materials
2 x 50 mL R1 and 2 x 12.5 mL R2 – 50 assays (manual) / ≥ 500 assays (auto-analyzer)

For *in vitro* use only
Store between 2 - 8 °C

Method

Enzymatic UV test with urease and glutamate dehydrogenase (GlDH). The result comprises the sum of ammonia after urea cleavage and free ammonia. For differentiation, free ammonia must be determined in a separate test using E8390 Enzytec™ *Liquid* Ammonia and subtracted from the result of this test E8395 Enzytec™ *Liquid* Urea/Ammonia.

Principle

The enzyme urease cleaves urea to ammonia and carbon dioxide:

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

Ammonia reacts with α-ketoglutarate in the presence of GlDH and nicotinamide adenine dinucleotide (NADH) to form L-glutamate and NAD^+:

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GlDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The NADH consumption is stoichiometric with the amount of ammonia converted or half the amount of urea. This is measured by a decrease of absorbance at 340 nm.

Reagents

The reagents are ready-to-use.

- Reagent 1: 2 x 50 mL (buffer, NADH)
- Reagent 2: 2 x 12.5 mL (α-ketoglutarate, urease, GlDH)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C (see label). Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range (see test performance). The dilution factor must be taken into account in the calculation.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush and homogenize solid and semi-solid samples, extract suitable sample amount with perchloric acid and KOH.
- Milk samples: precipitate with trichloroacetic acid (e.g. 0.3 M, 1:4), centrifuge after approx. 5 min and use clear supernatant.
- Carrez clarification cannot be applied!
- Detailed sample preparation guideline available on request.

Assay procedure

Wavelength: 340 nm
Temperature: 37 °C or 20 - 25 °C
Measurement: Against air or against water
Sample: 8 - 170 mg/L

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate approx. 20 min at 20 - 37 °C. Read absorbance A_2.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

Calculation of results

Calculation of sample solutions

$$\Delta A = (A_1 \times df - A_2)_{sample} - (A_1 \times df - A_2)_{RB}$$

df: dilution factor
RB: Reagent blank

$$df = \frac{(\text{sample volume} + R1)}{(\text{test volume})} = 0.808$$

$$c_{Urea}\,[g/L] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times 2 \times d \times v \times 1000)}$$

V:	Test volume [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 60.06
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

For a determination at 340 nm this results in:

$$c_{Urea}\,[g/L] = 0.1239 \times \Delta A$$

Calculation of solid samples

$$\text{Content}_{Urea}\,[g/100\ g] = \frac{c_{Urea}\,[g/L]}{\text{weight}_{sample}\,[g/L]} \times 100$$

Differentiation of urea and free ammonia

$$c_{Urea\ w/o\ free\ ammonia}\,[g/L] = c_{Urea/Ammonia} - (c_{Ammonia} \times 1.763)$$

Performance data

Specificity

The test is specific for urea/ammonia and shows no side activities or interferences with relevant organic acids, sugars or preservatives such as ascorbic acid. Sulfite and citric acid do not interfere at or below 6.25 g/L and 25 g/L, respectively.

Linearity & Measuring range

Linearity is given up to 190 mg/L urea. The recommended measuring range is between 8 and 170 mg/L urea.

Sensitivity

The limit of detection (LoD) and the limit of quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution for a sample volume of v = 100 µL.
This results in an LoD of 4.0 mg/L and an LoQ of 8.0 mg/L.

For a maximum sample volume of v = 1000 µL and a test volume of V = 3.5 mL, theoretical LoD and LoQ values were determined by calculation according to Lambert-Beer.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005, resulting in an LoD of 0.08 mg/L.
Based on ΔA = 0.020, an LoQ of 0.33 mg/L was calculated.

Automation & Validation reports

Application sheets for automated systems and customer validation reports are available on request.

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