Enzymatic assay for the determination of 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 glutamate dehydrogenase (GlDH).

Principle

Ammonia ($NH4^+$) reacts with α-ketoglutarate in the presence of (GlDH) and reduced nicotinamide-adenine dinucleotide (NADH), to form L-glutamate and NAD^+.

α-ketoglutarate + $NH4^+$ + NADH —GlDH→ L-glutamate + NAD^+ + H_2O

The NADH consumption is stoichiometric with the amount of ammonia which is measured by the 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 / GlDH)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. 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)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Crush and homogenize solid and semi-solid samples, weigh suitable sample amount into a volumetric flask and extract with perchloric acid; adjust pH value to pH 7 with KOH, fill up to final volume.
- For fat separation, allow sample solution to cool down (e.g. 20 min in the refrigerator), remove the fatty layer and filter the aqueous part.
- Milk samples: Mix 1 ml milk + 4 ml trichloroacetic acid (0.3 M), after approx. 5 min centrifuge the sample and use the clear supernatant in the test.
- Neutralize strongly alkaline (> pH 10) or strongly acidic (< pH 3) samples with KOH / NaOH or HCl.

Assay procedure

Wavelength:	340 nm
Optical path:	1 cm
Temperature:	37 °C / 20 - 25 °C
Measurement:	Against air or against water
Sample:	5 - 95 mg/l

	Reagent blank	**Samples / Controls**
Reagent 1	2000 µl	2000 µl
Sample / Control	-	100 µl
Dist. water	100 µl	-
Mix, incubate for 1 min at 37 °C or 3 min at 20 - 25 °C. Read absorbance A_1, then add:		
Reagent 2	500 µl	500 µl
Mix, incubate 5 min at 37°C or 10 min at 20 - 25 °C. Read absorbance A_2.		

The reagent blank must be performed 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

$$df = \frac{(\text{sample volume} + R1)}{(\text{sample volume} + R1 + R2)} \times 100 = 0.808$$

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

V:	Total volume [ml]	= 2.600
MW:	Molecular weight [g/mol]	= 17.03
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:

$$\mathbf{c_{Ammonia}\,[g/l] = 0.0703 \times \Delta E}$$

Calculation of solid samples:

$$Content_{Ammonia}\,[g/100\,g] = \frac{c_{Ammonia}\,[g/l]}{weight_{sample}\,[g/l]} \times 100$$

Notes

- Carrez clarification cannot be used in sample preparation due to the absorption of ammonia.
- Due to the volatility of ammonia, it is recommended that Reagent 1 is added first and then the sample amount should be pipetted.

Performance data

Specificity

The test is specific for ammonia and shows no side activities or interference with various relevant acids, sugars or preservatives such as sulfite.

Linearity & Measuring range

Linearity is given up to 100 mg/l ammonia. The recommended measuring range is between 5 and 95 mg/l ammonia.
If this range is exceeded, the samples should be diluted with dist. water to an ammonia concentration within the measuring range. The dilution factor must be taken into account in the calculation.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11 in buffered aqueous solution:

- LoD = 0.7 mg/l
- LoQ = 1.2 mg/l

Automation

Application sheets for automated systems are available on request.

Disclaimer

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