UV test for the determination of ammonia in foodstuff and other sample material
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C (36 - 46 °F)

This test was evaluated using selected samples of the following matrices: milk, fruit juices, wine, cheese, and processed meats and meat products.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

Ammonia (NH_4^+) reacts with α-ketoglutarate in the presence of glutamate dehydrogenase (GlDH) and reduced nicotinamide-adenine-dinucleotide (NADH) to form L-glutamate and NAD^+:

α-Ketoglutarate + NH_4^+ + NADH $\xrightarrow{GlDH}$ L-glutamate + NAD^+ + H_2O

The amount of NADH formed is proportional to the amount of ammonia formed and is measured at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however, it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, NADH
- Reagent 2: 2 x 12.5 mL with buffer, α-ketoglutarate, GlDH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & Shelf Life

If stored as directed and between 2 to 8°C (36 – 46°F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & Disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample Preparation

- Manual and automated sample preparation is the same.
- Samples must be at room temperature for testing.
- Clear, and nearly neutral liquid samples may be used directly or to obtain dilution within the test range (see Performance data) after dilution with distilled water.
- Strong acid or alkaline samples must be neutralized with KOH or HCl to a pH value of 6 to 8.
- Cloudy samples must be filtered or centrifugated.
- Samples containing CO_2 or carbonic acid must be degassed before use.
- Crush and homogenize solid or semi-solid samples and extract with water (e.g., 30min at 60°C). To clarify, filter, centrifuge, or, if necessary, use perchloric acid.
- Highly fatty samples must be weighed into a volumetric flask and extracted with hot water. Allow extract to cool to for fat separation (e.g., through a 15 min ice bath). Fill the flask to the calibration mark with water and then filter the aqueous solution before testing.
- **Important: Do not** perform Carrez clarification with this test because Carrez reagents absorb ammonia. Use perchloric acid instead to clarify high protein samples.

3.1. Milk

- In a screw cap centrifuge tube, add 4ml trichloroacetic acid (0.3 M) to 1 ml of milk.
- Thoroughly mix fully and then incubate for 5 min. Then centrifuge 3 minutes at 4000 rpm.
- Decant the supernatant after centrifugation and neutralize with KOH (5 M), bring to a known volume, e.g. by transferring in a volumetric flask, filter and use at least 500 µL of the clear supernatant for measurement; this extract can also be used for determination of urea.

3.2. Cheese, Processed Meats, and other Meat Samples

- Homogenize solid cheese or meat samples and weigh 5 g into a centrifuge tube with screw cap.
- Add 20 ml of 1 M perchloric acid and mix for 2 min until uniform suspension is obtained.
- Transfer the content of the beaker quantitatively with additional water into a 100 mL volumetric flask and fill up with water to 100 mL so that the aqueous phase will be at the calibration mark (fat layer will be above the calibration mark).
- Store the flask between 2 to 8 °C for 20 min in a refrigerator to precipitate fat and perchlorate.
- Filter to obtain a clean supernatant and use this solution (dilution may be necessary) in the assay.

4. Test performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (68 - 99°F) (during measurement)
Measurement: against air (without cuvette)
Measuring range: 4 - 80 mg/L

	Reagent blank	**Sample / control**
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 addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **20 min at 20 - 37 °C** and read absorbance A_2.		

4.1. Application notes for Test performance

- The reagent blank value (water sample) must be determined **once for each run** and subtracted **from each** sample result.
- Specified incubation times were verified and established **at 25°C (77 °F).** The test may generally perform within a range between **20 - 37°C (68 - 99°F)**.
- Due to the volatility of ammonia, it is advisable to first add Reagent 1 and only then to pipette the sample.
- Use separate pipette tips for each sample extract or control solution to avoid cross-contamination. Pre-rinse each pipette tip before dispensing.

- Multistepper pipettes are recommend for adding Reagent 1 or 2. Use a separate tip for each reagent.
- Wait for the reaction to complete or for absorbance readings to stabilize (at least during the first test runs or validation). If absorbance does not stabilize within the recommended incubation time, measurements should continue at intervals of, for example, 5 minutes until a constant extinction value can be achieved.
- To obtain acceptable analytical precision, the measured rate of absorbance decrease (ΔA) should generally be at least 0.100 units or larger.
- If the measure rate of absorbance decreases too small (< 0.020), the sample solution must be prepared again with a larger sample or a lower dilution factor.
- If the extinction difference of the samples is very large (e.g., > 1.500), the sample solution may need to be diluted.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of ammonia

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

df: Dilution factor
RB: Reagent blank

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

The specified dilution factor (df) of 0.808 applies to a **basic application** of **100 µL**. Increasing the sample volume is possible (maximum 1000 µL – refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.
Increases in the sample volume may influence test performance. Therefore, verify each matrix. Adjust the **reagent blank** value to match the **modified sample volume.**

$$\mathbf{C_{ammonia}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.0703 \times \Delta A} \times F$$

If the sample extract was diluted prior to measurement, this result must be multiplied by the **pre-dilution factor F.**

V:	Test volume basic application [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)

5.2. Calculation of solid samples

When analyzing solid and semi-solid samples that are weighed for extraction, the content is related to the weighed amount:

$$Content_{ammonia}\ [g/100\ g] = \frac{C_{ammonia}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ sample\ solution]} \times 100$$

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Recovery of aqueous control solutions must be within 100 ± 5 %. We recommend the use of certified reference materials or standard solutions, such as:

- LGC reference standard: Ammonia ion in H_2O; c = 10 mg/L NH_3 (Article No. VHG-INH3-10-100)
- LGC reference standard: Ammonia ion in H_2O; c = 100 mg/L NH_3 (Article No. VHG-INH3-100-100)

The following material can be used to produce a control or spike solution:

- Ammonium sulfate, $(NH_4)_2SO_4$, p.a. (e.g. Carl Roth Article No. 198271027).

A stock solution with a concentration of c=1g/L NH_3 can be prepared as follows:

- Molecular weight of $(NH_4)_2SO_4$ = 132.14 g/mol
- Molecular weight of NH_3 = 17.03 g/mol
- Molar ratio = 1 : (7.759/2) → conversion Factor= 3.8795

3.8795 g of $(NH_4)_2SO_4$, dissolved in 1 L of distilled water, corresponds to a concentration of 1 g/L NH_3.

This stock solution can be diluted further with distilled water or to spike liquid matrices.

6. Performance data

6.1. Specificity & Side Effects

The test is specific for ammonia. During validation, a selection of substances with the potential for interference were evaluated (See Validation Report for details). No cross-reactions or other interfering effects were observed for the tested substances.

6.2. Interferences & side activities

The test evaluations of the following potentially interfering substances showed no interference effects in the presence of ammonia at or below the concentrations indicated in parentheses:

D-Glucose (150 g/L), D-Fructose (80 g/L), D-Galactose (0.5 g/L), Sucrose (0.5 g/L), Lactose (0.5 g/L), Glycerol (0.2 g/L), D/L-Malic Acid (10 g/L), D/L-Tartaric Acid (5 g/L), Citric Acid (2.5 g/L), D-Gluconic Acid (0.2 g/L), D- and L-Lactic Acid (0.15 g/L), Acetic Acid (5 g/L), Ascorbic Acid (2.5 g/L), Sulfite (2.5 g/L), Potassium Nitrate (0.25 g/L), Sodium Nitrite (0.05 g/L), Sodium Chloride (50 g/L)

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 80 mg/L ammonia, with the recommended measuring range between 4 and 80 mg/L (sample volume of 100 µL).

The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 0.8 mg/L. The limit of quantification (LoQ) was determined by precision profile and is 4 mg/L.

The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.04 mg/L. Based on ΔA = 0.010, an LoQ of 0.08 mg/L was calculated.

7. Supporting Documents

The following documents are available upon request:

- Enzytec™ *Liquid* Ammonia E8390 Validation Report
- Enzytec™ *Liquid* General Sample Preparation Guide
- Enzytec™ *Liquid* Ammonia E8390 Excel Calculation Templates
- Enzytec™ *Liquid* Ammonia E8390 Technical Information
- Enzytec™ *Liquid* Troubleshooting Guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Method Limitations

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

10. Limitation of Liability

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