AMMONIA
(LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007403
K-AMIARLQ

04/24

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

INTRODUCTION:

Ammonia is a widely occurring natural compound, often produced as a consequence of microbial protein catabolism, and thus serves as a quality indicator of fruit juice, milk, cheese, meat, seafood and bakery products. Unlike other products, this kit benefits from the use of a recombinant glutamate dehydrogenase that is not inhibited by tannins found in, for example, grape juice and wine.

PRINCIPLE:

In the presence of glutamate dehydrogenase (GlDH) and reduced nicotinamide-adenine dinucleotide phosphate (NADPH), ammonia (as ammonium ions; $NH4^+$) reacts with 2-oxoglutarate to form L-glutamic acid and $NADP^+$ (1).

(1) 2-Oxoglutarate + NADPH + NH_4^+ $\xrightarrow{\text{(GlDH)}}$ L-Glutamic acid + $NADP^+$ + H_2O

The amount of $NADP^+$ formed is stoichiometric with the amount of ammonia. NADPH consumption is measured by the decrease in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for ammonia (NH_3 / NH_4^+).
- The limit of detection (LOD) is 0.001 g/L, and the limit of quantification (LOQ) is 0.003 g/L (using a sample volume of 0.1 mL).
- The recommended working range is between 0.004 and 0.080 g/L (using a sample volume of 0.1 mL). This corresponds of 0.4 to 8 µg of ammonia per assay.

INTERFERENCE:

No interfering compounds have been identified.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents can be disposed of with the laboratory waste.

NOTE: For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KIT CONTENTS:

Kit suitable for manual and automated formats. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL): NADPH
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Reagent 2 (2 x 12.5 mL): GlDH
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Standard (5 mL): Ammonia standard (0.08 g/L).
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

NOTE: The ammonia standard solution is only assayed if there is some doubt about the accuracy of the spectrophotometer being used or if it is suspected that inhibition is being caused by substances in the sample. The concentration of ammonia is determined directly from the extinction coefficient of $NADP^+$.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25 °C) before use.

MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 2.60 mL
Sample solution: 0.004 g/L to 0.08 g/L (i.e. 0.4 - 8 µg of ammonia per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 mins at 20 - 37°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~5 mins at 20 - 37°C, then read the absorbances (A_2).**		

* either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 min intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

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

CALCULATION:

NOTE: These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) of the optical densities due to the reagent volume.

$$df = \frac{\text{Sample volume [mL]+ R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the ammonia manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{ammonia}$

The value of $\Delta A_{ammonia}$ should as a rule be at least 0.1 absorbance units to achieve sufficiently accurate results.

$$\Delta A_{ammonia} = (A_1 \times df - A_2)_{sample} - (A_1 \times df - A_2)_{blank}$$

It follows for the ammonia manual assay procedure:

$$\Delta A_{ammonia} = (A_1 \times 0.808 - A_2)_{sample} - (A_1 \times 0.808 - A_2)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor. If volumes are changed, the performance may be affected.

3. Calculation of the ammonia content

The concentration of ammonia can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{ammonia} \quad [g/L]$$

where:

V = final volume [mL]

MW = molecular weight of ammonia [g/mol]

ε = extinction coefficient of NADPH at 340 nm [l x mol^{-1} x cm^{-1}]

d = light path [cm]

v = sample volume [mL]

It follows for the ammonia manual assay procedure:

$$c = \frac{2.6 \times 17.03}{6300 \times 1.0 \times 0.1} \times \Delta A_{ammonia} \quad [g/L]$$

$$= 0.07028 \times \Delta A_{ammonia} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

4. Calculation of the ammonia content in solid or semi-solid samples:

When analysing solid and semi-solid samples which are weighed for sample preparation, the content (g/100g) is calculated from the amount weighed as follows:

$$\frac{c_{ammonia}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ \ sample\ solution]} \times 100 \quad [g/100g]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for biochemistry analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

NOTE: For each batch of samples that is applied to the determination of ammonia a calibration curve must be performed concurrently using the same batch of reagents.

Parameter	Details
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1-3 min [20 - 37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Incubate 5 mins at [20 - 37°C] – Measure A_2 at 340/405 nm – Calculate $A_1 – A_2$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 0.08 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.01 g/L (dilute Standard 8-fold) Calibrator 3 0.04 g/L (dilute Standard 2-fold) Calibrator 4 0.08 g/L (use Standard as-is) *Perform all dilutions with distilled water.*

SAMPLE PREPARATION:

1. Sample dilution

The amount of ammonia present in the sample should range from 0.004 g/L to 0.08 g/L. If the value of $\Delta A_{ammonia}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value $\Delta A_{ammonia}$ is too high (e.g. >1.3), increase the dilution in distilled water.

Dilution Table

Estimated Concentration of ammonia (g/L)	Dilution with Water	Dilution factor (F)
<0.08	No dilution required	1
0.08 – 0.8	1mL sample + 9 mL water	10
0.8-8.0	1mL sample + 99 mL water	100

2. General sample preparation guide

IMPORTANT NOTE: Carrez clarification should not be used in the sample preparation for ammonia determination as it negatively impacts the assay performance.

- Clear, slightly coloured and approximately neutral, liquid samples at a concentration up to 0.08 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH <3.0) should be neutralised to approximately pH 8.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenised, extracted in water and filtered or centrifuged if necessary.
- Strongly coloured samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinise samples by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Centrifuge (at 1,500 g for 10 minutes) or filter and neutralise the supernatant with 1 M KOH. Alternatively follow sample preparation example (c) below.
- Remove fat if needed by extracting the sample with hot water (>60°C). Weigh 5 g of sample into a glass beaker and add approximately 60 mL of hot distilled water with stirring. Equilibrate to room temperature and quantitatively transfer to a 100 mL volumetric flask. Fill the volumetric flask to the mark with distilled water. Store on ice or in a refrigerator for 15-30 minutes and then filter.

3. Suggested sample preparation examples

(a) Determination of ammonia in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g.
Typically, a 2-fold dilution in distilled water is required.

(b) Determination of ammonia in fruit juice (e.g. apple juice). Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of juice for 5 minutes at 15,000 g.
Typically, no dilution is required.

(c) Determination of ammonia in solid cheese and meat products (e.g. mince meat and brie cheese). Homogenize solids samples in a blender and accurately weigh 5 g of representative material into a 100 mL Duran® bottle. Add 20 mL of 1 M perchloric acid and mix with a spatula for 5 minutes, or until the sample is evenly dispersed. Add 40 mL of distilled water and stir for another 10 minutes. Adjust the pH to 7.0 using 8 M potassium hydroxide, quantitatively transfer the suspension into a volumetric flask and make to 100 mL with distilled water. Store on ice or in a refrigerator for 20 minutes to precipitate potassium perchlorate and allow separation of the fat. Filter, discarding the first 3-5 mL, and use the clear filtrate for the assay.
Typically, no further dilution is required.

IMPORTANT NOTE: Users should perform in-house matrix validation prior to routine use. This process will highlight any problematic matrices encountered. The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found on the product page:

- Quick Reference Guide
- MegaCalc™
- Safety Data Sheets (SDS)
- Certificates Of Analysis (COA)
- Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents. It is the user's responsibility to perform in-house matrix validation work prior to routine use.