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Product Information

Ammonia Assay Kit

Catalog Number **MAK310**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Ammonia (NH_3) or the ammonium ion (NH_4^+) is found in the atmosphere, rainwater, soil, seawater, and volcanic areas. It is widely used as fertilizer, being an important source of nitrogen for living systems. It also plays a role in animal physiology. Ammonia is produced by amino acid metabolism and is converted to urea in the human liver through the urea cycle.

The Ammonia Assay Kit provides a simple and high-throughput adaptable assay for quantitative determination of ammonia/ammonium ion concentration in biological samples, such as urine, and environmental samples. This assay is based on the *o*-phthalaldehyde method in which the reagent reacts with ammonia/ammonium ion producing a fluorometric result (λ_{ex} = 360/λ_{em} = 450nm), proportional to the ammonia concentration in the sample.

The kit has a linear detection range of 0.012–1 mM ammonia in a 96 well format.

Components

The kit is sufficient for 200 assays in 96 well plates.

Ammonia Assay Buffer Catalog Number MAK310A	20 mL
Reagent A Catalog Number MAK310B	1 mL
Reagent B Catalog Number MAK310C	1 mL
NH_4Cl Standard Catalog Number MAK310D	400 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Allow all reagents to come to room temperature before use.

NH_4Cl Standard – Dilute 10 µL of 20 mM NH_4Cl standard with 190 µL of ultrapure water to prepare a 1 mM standard solution.

Storage/Stability

The kit is shipped at room temperature. Storage at –20 °C is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards and samples.

<u>NH_4Cl Standards for Fluorometric Detection</u>

Add 0, 25, 50, and 100 µL of the 1 mM NH_4Cl Standard Solution into wells of a 96 well plate. Add ultrapure water to each well to bring the volume to 100 µL, generating 0 (blank), 0.25, 0.5, and 1.0 mM standards.

<u>Sample Preparation</u>

Urine samples should be diluted 50-fold with ultrapure water prior to assay.

Samples should be clear and not contain any particles or precipitates. Particles or precipitates should be removed by centrifugation for 5 minutes at 14,000 rpm or by filtration.

Notes: This assay is compatible with most detergents, chelators, and buffer components. Proteins and primary amine-containing buffers such as Tris or glycine should be avoided.

Include the same concentration of the sample buffer in the standards and blank.

Assay Reaction

1. Add 10 µL of each standard into wells of a 96 well plate.
2. Add 10 µL of each sample into separate wells.
3. Prepare the Working Reagent Mix according to the scheme in Table 1. 90 µL of the Working Reagent Mix is required for each reaction (well).

Table 1.
Working Reagent Mix

Reagent	**Samples and Standards**
Ammonia Assay Buffer	90 µL
Reagent A	4 µL
Reagent B	4 µL

4. Add 90 µL of Working Reagent Mix to each sample and standard well. Immediately tap plate to mix.
5. Incubate the reaction for 15 minutes in the dark at room temperature.
6. Measure the fluorescence intensity (λ_{ex} = 360 nm/λ_{em} = 450 nm).

Results

Calculation

Note: A new standard curve must be set up each time the assay is run.

The background is the value obtained for the 0 (assay blank) NH_4Cl Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NH_4Cl standards to plot a standard curve and determine the slope.

$$[NH_3] \text{ (mM)} = \frac{F_{SAMPLE} - F_{BLANK}}{\text{Slope}}$$

F = Fluorescence intensity

Note: If ammonia concentration is >1 mM, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Appendix

Assay Procedure for Handheld Fluorimeter

1. Prepare 1 mM NH_4Cl Standard by mixing 5 µL of the 20 mM NH_4Cl standard with 95 µL of ultrapure water or sample buffer.

2. In separate mini-glass tubes, add 10 µL of ultrapure water or sample buffer (blank), 10 µL of 1 mM NH_4Cl Standard, and 10 µL of Sample. Then add 90 µL of Working Reagent Mix (90 µL of Assay Buffer, 4 µL of Reagent A and 4 µL of Reagent B) to each tube and mix. Incubate for 15 minutes in the dark.

3. Switch on the reader. To calibrate the reader, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Reader starts Measuring.

   Press "<-Std -> ", until the window shows "1.00".

   Place the 1 mM NH_4Cl Standard into the Sample holder. Press "Measure". The reader shows "Calibrate Finished". Press "Return".

   4. Place the sample tube into the sample holder.

   Press "Measure" → "Assay 1" → "Measure".

   The ammonia concentration (mM) will be displayed in the window. Record the data, or press "Save" to save the data for later retrieval. Press "Return" and then "Measure" for the next sample.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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