Ammonia Assay Kit

Catalog Number KA3772

200 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

- Application

✓ Ammonia/ammonium determination in biological (e.g. urine) and environmental samples.

- Features:

✓ Fast and sensitive. Linear detection range of 0.012 - 1 mM ammonia.

✓ Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Background

AMMONIA (NH_3) or its ion form ammonium (NH_4^+) is an important source of nitrogen for living systems and is ubiquitously present in the nature. Simple, direct and automation-ready procedures for measuring NH_3 are very desirable.

Principle of the Assay

The Ammonia Assay Kit y is based on an improved o-phthalaldehyde method. This reagent reacts with ammonia/ ammonium and forms a fluorescent product. The fluorescence intensity ($\lambda_{ex/em}$ = 360/450 nm) is proportional to the ammonia concentration in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	20 mL
Standard (NH_4Cl)	400 µL
Reagent A	1 mL
Reagent B	1 mL

Storage Instruction

Store kit at -20°C. Shelf life of 12 months after receipt.

Materials Required but Not Supplied

Pipetting devices, centrifuge tubes, black flat bottom 96-well plates and plate reader.

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Assay Protocol

Assay Procedure

- Assay Procedure for 96-Well Plate Reader

  Use black flat-bottom 96-well plates. Prior to assay, bring all reagents to room temperature.

  Note: (1). This assay is compatible with most detergents, chelators and buffer components. Proteins and primary amine-containing buffers (e.g. Tris, glycine) should be avoided, if possible. For best results, include the same concentration of the sample buffer in the standards and blank. (2). Samples should be clear and not contain any particles or precipitates. Particles or precipitates can be removed by centrifugation for 5 min at 14,000 rpm or by filtration. (3). Urine samples should be diluted 50-fold in water prior to assay.

1. Standards. Prepare 200 µL 1 mM Standard Premix by mixing 10 µL 20 mM NH_4Cl Standard and 190 µL H_2O. Dilute standards as follows.

No	Premix + H_2O	Standard mM)
1	100 µL + 0 µL	1.00
2	50 µL + 50 µL	0.50
3	25 µL + 75 µL	0.25
4	0 µL + 100 µL	0

   Transfer 10 µL standards into separate wells of the plate.

   Transfer 10 µL of each sample in separate wells of the plate.

2. Assay. Prepare enough working reagent for 4 standards and all samples. For each reaction combine the following: 90 µL Assay Buffer, 4 µL Reagent A and 4 µL Reagent B. Add 90 µL Reagent to all wells. Immediately tap plate to mix. Incubate for 15 min in the dark at room temperature. Measure fluorescence intensity at 360/450 nm on a plate reader.

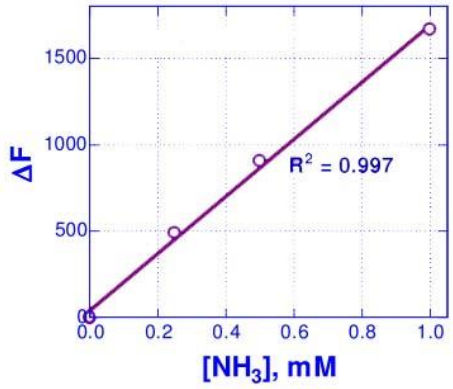
Data Analysis

Calculation of Results

Plot the ammonia standard curve and determine its Slope. The ammonia concentration of a Sample is calculated as

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

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and the blank (i.e. #4 H_2O), respectively. If ammonia concentration is higher than 1 mM, dilute Sample in water and repeat assay. Multiply the results by the dilution factor.



Standard curve performed on a 96-well plate reader;

Resources

References

1. Gips CH, et al (1970). Preservation of urine for ammonia determination with a direct method. Clin Chim Acta. 29(3):501-5.
2. Beecher GR, Whitten BK (1970). Ammonia determination: reagent modification and interfering compounds. Anal Biochem. 36(1):243-6.
3. Rodger MR, Jenkins P (1984). Enzymic fluorometric assay of plasma ammonia with a centrifugal analyzer. Clin Chem. 30(10):1670-2.