Technical Manual

FluoroDazzle Ammonia Assay Kit

Catalogue Code: BA0054

Pack Size: 200 assays

Research Use Only

DESCRIPTION

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

KEY 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.

APPLICATIONS

Ammonia/ammonium determination in biological (e.g. urine) and environ-mental samples.

KIT CONTENTS

Assay Buffer:	20 mL	**Standard:**	400 µL NH_4Cl
Reagent A:	1 mL	**Reagent B:**	1 mL

Storage conditions: This product is shipped at room temperature. Store kit at -20°C. Shelf life of 6 months after receipt.

Precautions: 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 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 360/450nm on a plate reader.

CALCULATION

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

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

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.

ASSAY PROCEDURE FOR CUVETTE-BASED FLUORIMETER

1. Standard. Prepare 1 mM Standard by mixing 5 µL of the provided 20 mM standard with 95 µL H_2O or sample buffer.

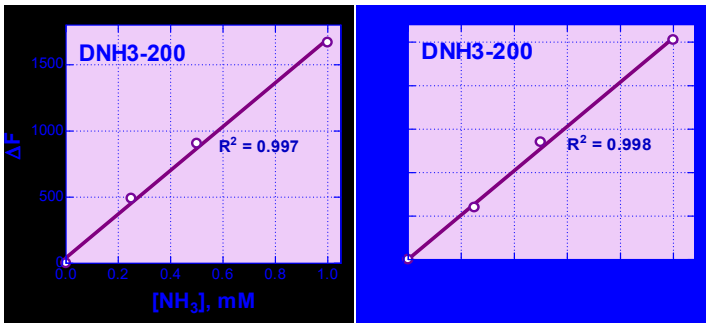
2. In separate mini-glass tubes, add 10 µL H_2O or sample buffer (Blank), 10 µL 1 mM Standard, and 10 µL Sample. Then add 90 µL Working Reagent (90 µL Assay Buffer, 4 µL Reagent A and 4 µL Reagent B) to each tube and mix. Incubate for 15 min in the dark.

3. Switch on and calibrate the reader by placing the "Blank" tube into the sample holder and read. Measure the 1 mM Standard to ensure the instrument has been calibrated.

4. Measure the samples and convert the ammonia concentration to (mM).

MATERIAL REQUIRED BUT NOT PROVIDED

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



Left: standard curve performed on a 96-well plate reader (Spectramax M2);
Right: correlation plot obtained on handheld fluorimeter (cat#: FL360450).

LITERATURE

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.

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