Ammonia/Ammonium Assay Kit (Fluorometric)

LS-K291-200 (200 Tests) • **Store at -20°C**

Introduction

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. This 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 environmental samples.

Components

Component	K291-200
	200 Tests
Assay Buffer	20 mL
Reagent A	1 mL
Reagent B	1 mL
Standard (NH_4Cl)	400 µL

Materials Not Supplied

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

Storage

This product is shipped at room temperature. Store kit at -20°C. Shelf life of 12 months after receipt

Ammonia/Ammonium Assay Kit (Fluorometric)

LS-K291-200 (200 Tests) • **Store at -20°C**

Assay Procedure

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.

Calculations

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

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

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.

Procedure for Handheld 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 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".

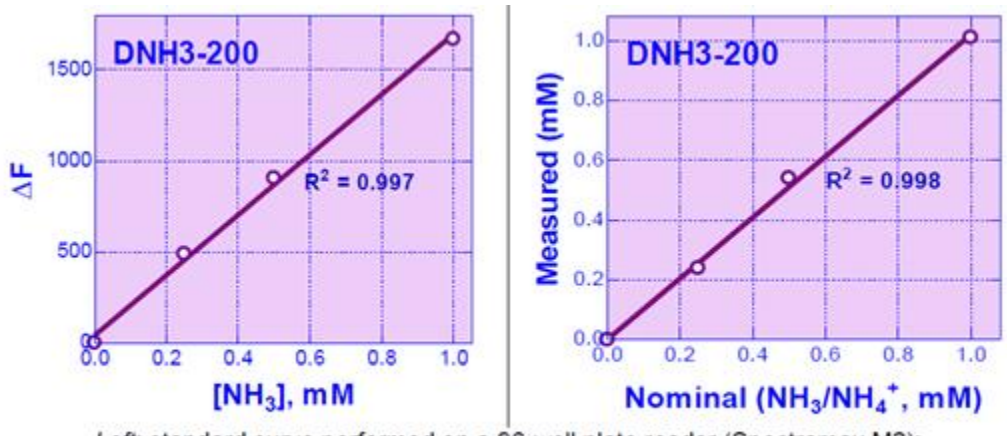
   Place the 1 mM Standard into the Sample holder. Press "Measure". The reader shows "Calibrate Finished". Press "Return".

4. Measure. 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.

Sample Data



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

Version: V.08.09.2018